Biological Aspects of Leather Manufacture

Proceedings of a seminar sponsored by the Central Leather Research Institute, Council of Scientific and Industrial Research, and held at the Institute on January, 31st to February, 2, 1968.

Editors

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FOREWORD

In recent years considerable thought is focussed on the various constituents of hides and skins, which in the tanned state constitute leather. This product, of biological origin has been the subject of a large number of investigations occupying the attention of workers engaged in diverse disciplines of science such as biology, biochemistry, chemistry, physics, technology, medical and animal sciences.

Keeping in mind, the advances made in the different aspects of this biopolymer, it was felt, that a seminar embracing (*i*) the biology of the raw hides and skins *vis-a-vis* their processing and the quality of finished leather, (*ii*) the factors affecting fibrous proteins both in health and disease, during the life of the animal and (*iii*) the role that microorganisms play both in the raw as well as the processed material and the related aspects, would contribute significantly and pave the way for further advancement. Hence a seminar on the " Biological Aspects of Leather Manufacture," was held at the Central Leather Research Institute from 31st January to 2nd February, 1968.

The seminar was inaugurated by Dr. A. Lakshmanaswamy Mudaliar, Vice-Chancellor of the University of Madras and a well known figure in the branch of medical science, under the presidentship of Dr. F. Verzar, Director, Institute of Gerontology, Basel, Switzerland, who has contributed much to the understanding of the ageing of collagen.

Thirty papers were presented both from India and abroad, in four sessions. The first session covered Microbiological aspects under the chairmanship of Professor T. S. Sadasivan, Director, Centre for Advanced Studies in Botany, and University Botany Laboratory, Madras. The second session on Hides and Skins was guided by Mr. L. Schroeder, Director, " Hude Centralen, " a cooperative organisation for the collection, curing, preservation and marketing of hides and skins, in Denmark. Professor G. N. Ramachandran, Director, Centre for Advanced Studies in Biophysics, University of Madras, was the chairman of the third session dealing with collagen. The final session on the aspects of veterinary Science and Animal Husbandry was guided by Dr. C.M. Singh, Director, Indian Veterinary Research Institute, Izatnagar. Popular lectures were delivered by Dr. G. Toth, Hungary and Dr. F. Verzar, Switzerland.

We record our deep indebtedness to the various authors from many countries who have presented papers, Chairmen of the different sessions and participants, for their contributions to this seminar.

It is hoped that this book covering the papers presented and the discussions that emanated, would be of great interest and use to technologists and workers in these fields of science.

Y. NAYUDAMMA,

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Microorganisms in the Tannery

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Laboratory studies have been carried out on the biodegradation occurring during the process of leather manufacture, right from raw to finish. Use of 0.05% sodium pentachlorophenate is recommended for the control of microorganisms in soaking above 22°C. *Penicillium aculeatum* and *Paecilomyces ehrlichii* were found to cause red spots on chrome tanned leather, and species of *Penicillium*, *Paecilomyces*, *Aspergillus* and *Verticillium* occurring on other leathers have also been recorded.

The problem of different effluents in the tannery have been studied from the point of view of their influence on fresh-water micro and macro fauna and results obtained were useful in solving the problem of purifying tannery effluents.

Leather, a biological material is composed of complex organic substances, especially proteins. It also contains fats, glycerides, minerals and water. A material of such a composition forms a favourable medium for the growth of microorganisms often resulting in great damage to it. Damage caused by microorganisms occurs at all stages of the manufacturing process — from the soaking stage upto the finished leather.

The present work is by no means an exhaustive summary of all microbiological damages. Our task consisted in studying the microflora which occur in the individual technological processes, to get acquainted with the ecological conditions suitable for the growth of the individual microbial strains and groups ; to find eventually the causes of various defects of biological origin on treated leather, and to propose suitable measures for avoiding the disastrous effects of microorganisms on hides, skins and leathers.

In addition to hides, skins and leathers, some tannery auxiliaries too (oils, greases, dilute solutions of tanning agents, protein binders, etc.) are suitable substrates for the growth of microorganisms ; on the other hand, some materials used in tanning technology have an adverse influence on the growth of microorganisms. This property is observed in sulphide-lime liquors which act through their high alkalinity ; the inhibitory effect of the sulphide present is well known. Chromium compositions are analogous to sulphide. Tanning agents too, especially in fresh or more concentrated solutions, retard the growth of microorganisms, particularly bacteria. Nevertheless, their effect varies greatly and depends on whether they are of vegetable or synthetic origin as well as on the type of tannin. While greatly diluted and exhausted vegetable tanning baths provide microorganisms with suitable sources of nourishment, especially by fermentation of polysaccharide non-tannin components, the vital processes of bacteria are inhibited in solutions of fresh tanning agents, by the presence of tannins.

The above substances are able to limit to a large extent the growth of living organisms when tannery waste-waters are let into a receptacle ; eventually, they may interfere with various methods of biological purification of tannery effluents.

Due to the fact that this is a global information concerning the work of our laboratory team, the author, instead of mentioning the individual problems in detail, summarises only the results obtained.

Materials and Methods

The proteolysis of hides and skins during soaking has been studied by the method previously described[1]. The materials and the methods used in our study of the damages produced in vegetable tanning[2], in chrome tanning[3], and the microbial damages occurring in drying leathers[4] and in finished leathers[5,6] are described, in the cited literature.

In our work on toxic influence of tannery waste-water and the individual chemicals and ingredients on the micro-organisms *Bacillus megatherium* and *Staphylococcus aureus*, we have used the method of growth curves including the determination LD_{50}, [7,8,9] and, in some instances, the Warburg manometric method. For determining and evaluating the noxiousness of waste waters from the individual tannery operations, on microorganisms, *Tubifex* sp., *Daphnia magna* and *Lebistes reticulatus*, the values of lethal, toxic and innocuous limits[10,11] were used.

Results and Discussion

Soaking : One of the factors which can adversely influence the course of soaking and, consequently, the quality of leather is the number and the species of microorganisms present in soaking water. A deleterious influence is observed especially with proteolytic bacteria which destroy the skin substance by means of proteases (peptidases). Growth of bacteria is promoted also by higher temperature (above 22°C) or by a longer period of soaking (over week-ends and during holidays). Under optimum conditions, proteolytic bacteria grow considerably, attack and digest the skin substance to the extent of producing holes. In the laboratory tests, we used dry salted Indian-goat-skins and native medium weight cow-hides. During soaking, spontaneous growth of bacteria occurred so that, after 40-48 hr, the flesh side of the goat-skin got slimy and gave off a putrid odour ; three days later there appeared putrefied cavities which deepened towards the grain side until perforation occurred, in many cases on the same day. Perforation of cow-hides developed in the very same way but within 5-7 days. The perforation of goat-skins began from the flesh side, while that of cow-hides occurred from the grain-side.

We isolated from the soak water and the putrefied spots, 6 strains of bacteria causing proteolytic perforation of raw-material, although they were used in model experiments as pure cultures. The isolated bacteria were determined as *Bacillus subtilis*, *B. megatherium*, *B. anthracoides*, *B. pumilus* and *Pseudomonas aeruginosa*.

For hindering effectively the perforation, we studied the effect of some bactericidal substances added to the soaking water. The results in Table 1 were evaluated on the fifth day for goat-skins and on the seventh day for cow-hides, when control samples were thoroughly perforated and eventually dissolved due to the enzymes. The table indicates that the most efficient *antibiotics* have not yet found application in practice. Sodium pentachlorophenolate and o-Cl-o-cresol from the cresol group seem to be highly efficienta nd technically accessible. The results show the possibility of avoiding putrefaction in soaking. It is necessary that the regular addition of disinfectants becomes part of the process, especially in summer, or when hides and skins remain soaked for longer periods.

TABLE 1

Effective concentration of Bactericides in soak waters

Bactericide	Concentration %	Quantity in g. for 1 m 3 water
Sodium p-nitrophenolate	0.15	1,500
Tetramethylthiuramdisulphide sodium	0.25	2,500
p-Cl-m-cresolate	0.08	800
o-Cl-o-Cresol	0.05	500
Sodium pentachlorphenolate	0.03-0.05	300-500
Sodium hexaflurosilicate	0.10	1,000
Chlorseptol	0.25	2,500
Tetracycline	0.001-0.0015	1-15
Oxytetracycline	0.001-0.0015	1-15
Chlortetracycline	0.001-0.0015	1-15
Chloramphenicol	0.001-0.0015	1-15
Streptomycin	0.001-0.0015	1-15

The next operation is liming and deliming where there is no biodeterioration of skin substance under the existing production conditions.

Vegetable tanning : The most common defect occurring in vegetable tanning comprises the so-called white spots (Fig. 1) which were supposed to

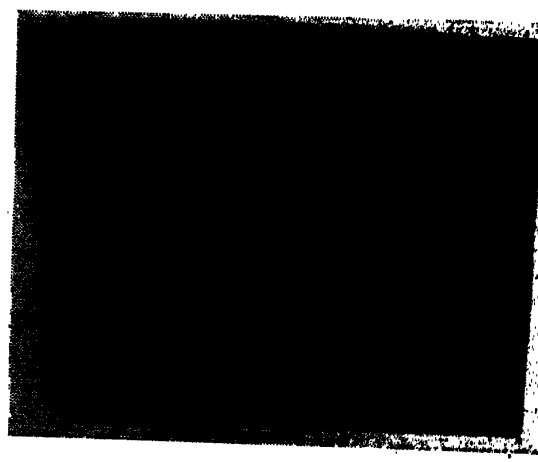

Fig. 1. *White spots on vegetable tanned leathers*

be caused by slimy fermentation of tan liquors. Their occurrence was explained by the formation of colonies of slime-producing bacteria on the grain-side, which hinder the penetration of tannins inwards. We have isolated the cause of this fermentation process, which has been identified as *Aerobacter aerogenes*[12], and literature mentions several of these agents (*e.g.*, *Leuconostoc.*) In any case, we have not succeeded in producing these white spots artificially although we got with a pure culture in our experiments, considerable slime-production in the vegetable tan liquor. On inoculating hides or skins with bacterial cultures which did not produce any slime isolated from the raw material, from soaking water and from vegetable tan liquor, no white spots appeared during tanning. On the other hand, when working during the in-plant tests of tanning leathers in sterile vegetable tan liquors, spots occurred very regularly. Due to this fact, after having carried out the critical analyses of the results obtained, we abandoned the opinion that the white spots are caused by slime fermentation of vegetable tan liquors. On the basis of various histological and chemical tests for proving the presence of slime matters, calcium and fatty acids in leathers and vegetable tan liquors, we concluded that the white non-tanned spots were not of biological origin and were caused by the less soluble calcium compounds. These results were confirmed experimentally using the so-called agar-diffusion analyses.

On the basis of the results obtained, a method was proposed for removing and preventing these white spots, by introducing an efficient method of deliming pelt using either the existing means or boric acid.

Chrome tanning : A frequent phenomenon in the tannery is the formation of the so-called red spots on chrome-tanned leathers. These leathers show a grey-green mycelium which grows and successively affects more and more of the leather surface. In the next stage of their development, the fungus starts forming a red pigment which is not removable from the leather. At first, this pigment has a dull appearance and its colour often penetrates upto the finish (especially in white leathers).

When studying red pigment, we noted that this pigment is partly soluble in water, acetone and butanol, insoluble in chloroform and ether. This pigment is extracellular and its red colour in acid medium changes into yellow in an alkaline medium. After having isolated and grown on artificial nutritious substrates of agar-type, fungus of the *Penicillium aculeatum* type (Raper and Fennel[12]) was identified (Fig. 2). After cultivation for several weeks on nutritious substrata and leathers, it forms sclerotia of lemon-yellow colour.

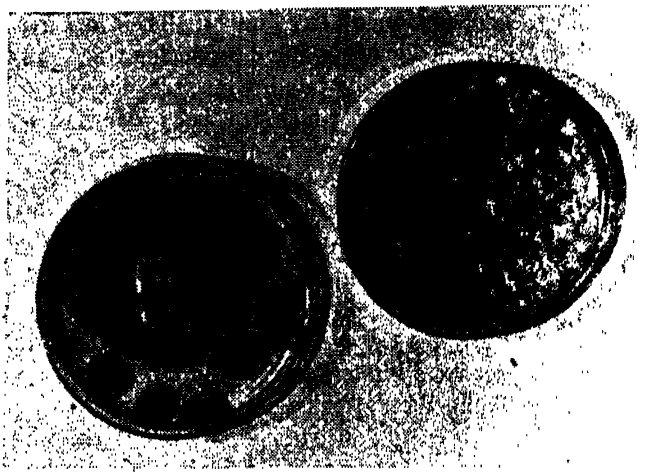

Fig. 2 *Penicillium aculeatum* — one of the originators of red spots on chrome tanned leathers.

We have to add, that the red colour of the chrome tanned pelt is not limited to a certain type of raw-material; this fungus attacks and causes red colour in box-sides, horse, chevreau, and pig-skin splits. Since chrome tanned goat-skins have been imported in this country from India, there has been a problem of red colour occurring in leathers from imported Indian chrome-tanned goat skins. Although these leathers are conserved by means of Preventol, we often encounter leathers having distinct red spots on their grain and flesh sides. After having isolated in pure cultures on Czapek-dox agar and after the analyses of the fungus grown on leather, we identified the cause of red colour as *Paecilomyces ehrlichii* (Delitsch *et* Henneberg[13]), which produces on firm nutrient medium a mycelium in brown or brown-green shades and a red to brownish-red pigment.

After having identified the origin of red colour, we passed on to resolving the question of how to avoid the injuries of this kind. Besides dipping chrome tanned leathers in fungicidal solutions, adding a fungicidal material to the tanning solution in the drum, also gives the required effect. Addition of sodium pentachlorphenolate 0.1—0.2% or 0.35% formaldehyde to the tanning bath before ending the tanning process is very useful (both values are based on the volume of float).

Drying of leathers : The microorganisms for their growth, need besides the substratum (leather), suitable humidity and temperature. If temperature and moisture rise inside the drying rooms or when air circulates too slowly, or the drying process takes too much time, there is a rapid growth of fungi (evidenced from the examples mentioned).

Technical leather for sports articles (chrome tanned side leather) in yellow colour was covered with dull green spots which penetrated the leather from the grain to the flesh side ; it was impossible to remove them by means of organic solvents (Fig. 3). By microbiological analysis we found that it was due to a fungus *Verticillium glaucum* (Bonord[13]), which is noted for attacking the substrates of higher grease content.

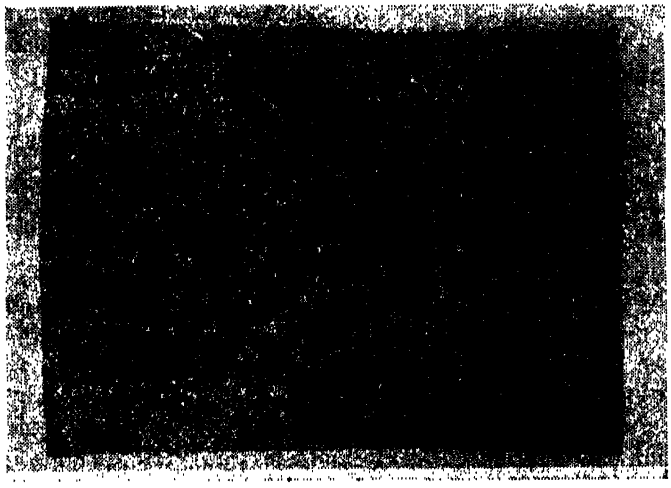

Fig. 3 Technical leather for sports articles. Spots originated by *Verticillium glaucum*

A quite analogous case occurred in the production of vegetable-tanned sole leather. On the light-brown substratum of the vegetable-tanned leather there were greenish spots and smudges visible from the grain and flesh sides which were also caused, by the growth of the fungus *Verticillium glaucum.*

Another instance is that of beige technical leather for sports articles which showed spots of various sizes and shapes in yellow-brown, dark-brown, grey and brownish green colours. As causes of these injuries, the following fungi were isolated : *Aspergillus ochraceus, A. wentii, Penicillium rugulosum, P. funiculosum* and *Paecilomyces varioti.* These kinds of fungi, however, are not the only source of injuries in drying, because the microbial degradation of leathers, can be caued by a far larger spectrum of fungal types. To avoid the formation of spots and the deterioration of the leather quality, it is necessary to carry out drying carefully and with a sense of responsibility. Delicate leathers can be conserved by the addition of a fungicide which is introduced into leather during fat-liquoring.

Finished Leathers: Major injuries to finished leathers are caused by fungi. In our studies on the occurrence of fungi in tanneries we identified mostly *Penicillium chrysogenum, P. luteum, P. brevicompactum, P. decumbens, P. rugulosum, P. aculeatum, P. funiculosum, Aspergillus niger, A. fumigatus, A. ochraceus, A. wentii, A. flavus-oryzae, Mucor mucedo, Rhizopus nigricans Paecilomyces varioti, Scoupulariopsis brevicaulis, Verticillium glaucum, Trichoderma* sp. [3, 4, 5, 6]. Many of the above fungi utilise for their growth and development the tanning substances so that we can find them on finished leathers as well as on the surface of vegetable tan liquors where they cause the fermentation of tanning by the action of the enzyme tannase.

The object of our work is to study the fungicidal substances which would be suitable for application on finished leathers and, after having carried out laboratory and confirmatory tests, to propose, the most suitable preservative together with the process for the in-plant application.

After having carried out screening tests, on a large scale, of antifungal substances, p-Cl-m-cresol and p-nitro-phenol were chosen for detailed tests. After having ascertained their inherent efficiency we studied the influence of fat-liquoring mixtures upon the efficiency of fungicides. We found that the fungicidal ability decreases with the increasing quantity of liquid substances in the medium ; p-nitrophenol which possesses a fungicidal ability in a medium without lipids at a concentration of 0.1% against *Aspergillus niger*, needs a concentration of 0.25% for being fungicidal in a medium with 15% fat.

The conservative efficiency of fungicides, incorporated into leathers, has been studied using several methods : cultivation on nutrient agar soil and storage in a medium with 100% RH at 37°C. The retention of fungicidal action has been followed after having stored the conserved samples for 4 months in running water, in soil, in storage rooms having different temperatures and freely exposed to the influence of summer weather. Some samples were placed in a ship which sailed in tropical zone within a year.

From the standpoint of the antifungal treatment, we have studied especially greased side leather, waterproof and technical leather, *e.g.* bicycle seats and camera cases. For practical use, we have proposed the following fungicidal agents :

greased side leather —0.2% p-nitrophenol

waterproof —0.35% p-nitrophenol

technical leathers (grease content upto 10%)
—0.2% p-nitrophenol.

At these concentrations, p-nitrophenol is well soluble in fat-liquoring mixtures and for this reason it is directly dissolved. The given percentage of the preservative refers to the leather weight, not to the fat-liquoring mixture weight.

Toxic influence of tannery chemicals and preparations

(*a*) *Influence of partial tannery waste-water* : At first, we studied a microbial population in waste-water from the different stages and the influence of these waters on aquatic fauna. In our microbiological studies we have noted especially the quantity of microorganisms of psychrophile and sporing type. In toxicological studies on aquatic fauna, we have assessed the lethal, toxic and innocuous limits (lethal limit — concentration which killed all tested organisms, toxic limit — concentration which caused perishing of a certain number of organisms ; innocuous limit — concentration without any distinct negative effect on tested organisms).

TABLE 2

Bacterial population of different tannery effluent-waters

Effluent	Psychrophile bacteria in 1 ml		Sporulating bacteria in 1 ml	
	1st day	14th day	1st day	14th day
Soaking	8.2×10^5	1.5×10^7	960	10100
Liming	$3.0 \times 10^{3*}$	0	168	0
Mechanical treatment and washing of pelt	3.8×10^3	3.7×10^4	240	290
Deliming	9.0×10^4	3.5×10^5	120	800
Bating	6.0×10^4	5.0×10^5	100	980
Pickle	2.5×10^4	1.2×10^3	94	30
Vegetable tanning	9.1×10^4	5.7×10^4	120	0
Washing after vegetable tanning	7.4×10^4	1.3×10^4	21	0
Chrome tanning	2.1×10^4	2.1×10^3	320	210
Washing after chrome tanning	1.7×10^4	1.3×10^4	260	150
Dyeing, greasing	2.0×10^4	1.5×10^5	180	1100
Total tannery waste water	3.2×10^4	9.7×10^4	670	480

*maximum quantity found.

TABLE 3

Influence of different tannery effluent-waters on some water macro fauna

Effluent		*Tubifex sp.*		*Daphnia magna*		*Lebistes reticulatus*	
		pits	drums	pits	drums	pits	drums
Soaking	L	non dil.	1 : 3	non.dil.	1 : 5	...	1 : 1
	T	1 : 3	1 : 5	1 : 2	1 : 10	...	1 : 2
	I	1 : 5	1 : 10	1 : 3	1 : 20	non dil.	1 : 5
Liming (pits)	L	1 : 100		1 : 100		1 : 40	
	T	1 : 150		1 : 150		1 : 150	
	I	1 : 200		1 : 200		1 : 60	
Liming (drums)		Sedim.	without sedim.	sedim	without sedim.	sedim.	without sedim.
	L	1 : 200	1 : 60	1 : 500	1 : 150	1 : 200	1 : 50
	T	1 : 600	1 : 150	1 : 800	1 : 200	1 : 300	1 : 60
	I	1 : 800	1 : 200	1 : 1000	1 : 300	1 : 400	1 : 80
Mechanical treatment and pelt washing	L	1 : 40		1 : 90		1 : 10	
	T	1 : 60		1 : 100		1 : 15	
	I	1 : 80		1 : 200		1 : 20	
Deliming	L	1 : 1		1 : 2		1 : 1	
	T	1 : 2		1 : 3		...	
	I	1 : 3		1 : 4		1 : 2	
Bating	L	non dil.		non dil.		1 : 1	
	T	1 : 2		1 : 5		1 : 2	
	I	1 : 3		1 : 10		1 : 3	
Pickle	L	1 : 5		1 : 5		1 : 5	
	T	1 : 15		1 : 10		1 : 7	
	I	1 : 20		1 : 15		1 : 10	
Vegetable tanning	L	1 : 30		1 : 30		1 : 100	
	T	1 : 100		1 : 100		1 : 200	
	I	1 : 150		1 : 150		1 : 300	
Washing after veg. tanning	L	1 : 30		1 : 30		1 : 600	
	T	1 : 400		1 : 200		1 : 700	
	I	1 : 500		1 : 300		1 : 800	
Chrome tanning	L	1 : 15		1 : 30		1 : 15	
	T	1 : 20		1 : 40		...	
	I	1 : 30		1 : 60		1 : 20	
Washing after chrome tanning	L	non dil.		1 : 1		1 : 1	
	T	1 : 2		1 : 3		...	
	I	1 : 3		1 : 5		1 : 2	
Dyeing, greasing	L	non dil.		1 : 10		1 : 5	
	T	1 : 4		1 : 40		1 : 10	
	I	1 : 5		1 : 60		1 : 20	
Total waste water	L	1 : 5		1 : 10		1 : 10	
	T	1 : 15		1 : 20		1 : 10	
	I	1 : 25		1 : 30		1 : 15	

L — Lethal limit, T — Toxic limit, I — Innocuous limit.

The results showed (Tables 2 and 3) that the most pernicious is the waste-water from liming which often remains thoroughly sterile and proved to be toxic for *Daphnia magna* when diluted to 1 : 800. The next in toxicity is waste-water from washing after vegetable tanning which has a great influence especially on higher water organisms and a dilution of 1: 800 is necessary to be innocuous for the fish *Lebistes reticulatus*. On the contrary, waste water from chrome tanning affects the microorganisms more, especially after a certain period of action. Innocuous limit for water organisms is attained with the 1 : 60 dilution. The totally mixed tannery waste-water proves to be toxic especially on microorganisms which shows the necessity for purifying the effluent water before letting into the recipient. The composition of the mixed waste-water varies considerably depending on the period of evacuation and for this reason its toxicity varies from stage to stage.

(*b*) *Influence of tannery chemicals.*—Because of the considerable contamination, the tannery waste-waters must be purified both mechanically and biologically. For this reason, it is necessary to know the admissible concentrations of harmful substances in these waters, so that the process of biological purification runs without any trouble. Using the method of growth curves studied especially the influence of sulphide and of trivalent and hexavalent chromium. For finally expressing the toxicity, we took the value of medium efficient concentration LD_{50} (TL_m-median tolerance limit), as described in other works[14,15,16] which deal with toxic influence on macro and microorganisms.

In comparison with data in literature we note that *Bacillus megatherium* is rather resistant against sulphide. Bringmann and Kuhn[17], mentioned an initial harmful concentration of 93 mg/l S" for *E. coli ;* 40 mg/l for alga *Scenedesmus* sp. Anderson[18] mentioned 4.1 mg/l S" for *Daphnia magna.* The above mentioned facts show a considerable fluctuation of values depending on the kind of test organisms and on the methodology of tests. It may be said that sulphide in concentrations upto 100 mg/l S" proves to be nontoxic for *Bacillus megatherium.* The mean efficient concentration of hexavalent chromium in $K_2Cr_2O_7$ is 75 mg/l, while LD_{50} for trivalent chromium in $K_2Cr(SO_4)_2$ 12 H_2O is almost double — 144 mg/l. The values quoted in literature vary considerably in dependence on the kind of test organism and the comparison shows, however, that *B. megatherium* is rather resistant to the action of chromium salts. However, we should admit that bacteria, not producing spores, can be influenced by the test substances to a larger extent than spore-producing bacilli and that lower LD_{50} values can be noted in the case of other kinds[19]. On the basis of pilot tests of biological purification of tannery waste waters carried out in our Institute and the influence of these waters on the recipient,

we are able to say that the quantity of 12 mg/l Cr^{III} determined in the mechanically purified waste water from tanneries has no negative influence on the development of aquatic biology both in purifying plant and in river.

TABLE 4

Mean effective concentration of some tannery chemicals on *Bacillus megatherium*

Chemical component	LD_{50} (mg/l)
$Na_2S\ 9H_2O$	1425
S''	190
$K_2Cr_2O_7$	217
Cr	76
$KCr(SO_4)_2\ 12\ H_2O$	1384
Cr^{III}	144

(*c*) *Influence of vegetable tanning agents* : For our tests we used the following tanning agents : Spruce (*Picea excelsa* Linn), oak (*Quercus* sp.), chestnut (*Aesculus hippocastanum* L.), quebracho (*Quebrachia lorentii*), *Schinopsis lorentii* and mimosa (family, *Leguminosae*). Since the extracts of these tanning agents contain, next to tannins, large quantities of non-tannins, we studied first which of these two components act on bacteria. A solution of non-tannins was obtained using a filtration method by passing the solution of tannin through the column of chrome hide powder. The solutions were sterilised by means of bacteriological filters and the strains *Bacillus megatherium*, *Staphylococcus aureus* and a mixture of psychrophile bacteria of city sewage were used as test-organisms. The influence of chestnut tannins in the respiration of *S. aureus* was studied using the Warburg manometric method.

The results showed that the toxic effects of tanning agents are caused by the presence of tannins, while non-tannins do not cause any reduction of bacterial numbers and do not possess any toxic ability. For final expression of the efficiency we chose the values LD_{50} TL_m which are given in Table 5.

TABLE 5

Influence of vegetable tanning agents upon microorganisms

Tanning agents	LD_{50} mg/l		
	Staphylococcus aureus	*Bacillus megatherium*	*Psychrophile bacteria* (mixture)
Chestnut	25.5	28.5	105.0
Mimosa	60.0	29.2	208.0
Quebracho	133.0	43.0	620.0
Oak	186.0	230.0	820.0
Spruce	340-0	1000.0	2800.0

All microorganisms studied are the most sensitive to the effect of chestnut tannins, the tannins of spruce being the least toxic. *B. methagerium* is very sensitive to mimosa and quebracho tannins too. The most resistant to tannins is a mixture of psychrophile germs of city sewage.

In the case of chestnut tannins which acted most on bacteria we studied the influence on the respiration of *S. aureus*. The results (Fig. 4) proved that

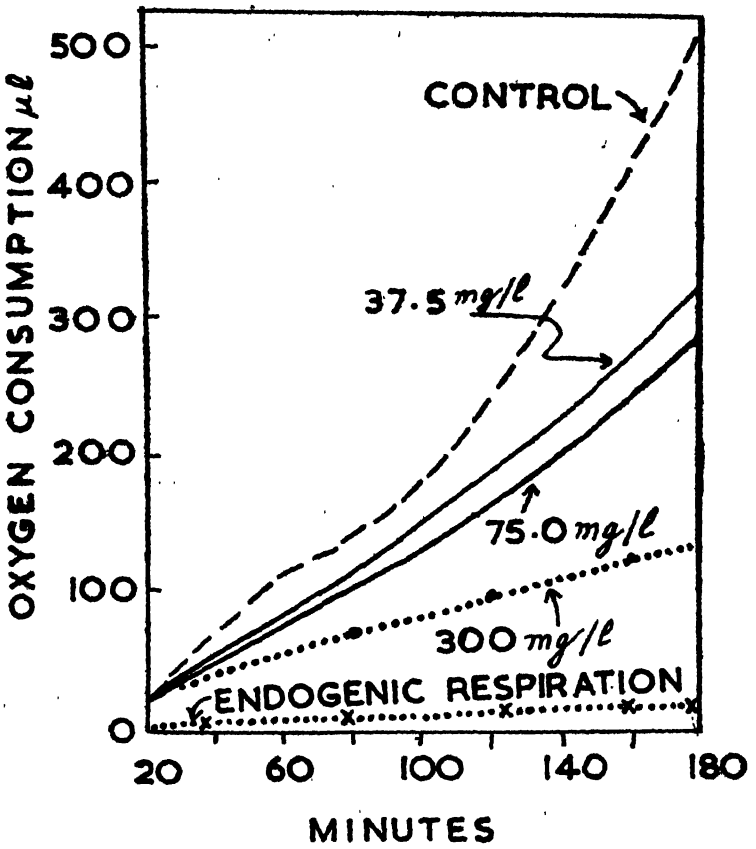

Fig. 4. Influence of chestnut tanning agents upon the respiration of *Staphylococcus aureus*

the concentration of 37.5 mgl. slows down breathing of bacteria. The values of metabolic quotients (Table 6) found after a 180 min. test are lower considerably in dependence on the increasing concentration of tanning agents.

TABLE 6

Influence of chestnut tanning agents upon the Respiratory Quotient (Qo_2) of *Staphylococcus aureus*

Tanning agent concentration	Qo_2 in ul	Qo_2 in %	decrease in %
Control	1241	100	...
37.5 mg/l	865	69	31
75.0 mg/l	789	63	37
300.0 mg/l	461	37	63

In the presence of 300 mg/l chestnut tannin the metabolic quotient was reduced by 63 percent.

The results lead to a conclusion, in accordance with Bronfenbrenner[20] who mentions the concentration reducing the respiration by 10 per cent to be bacteriostatical and the concentration reducing the oxygen consumption by 80 percent to be bactericidal.

(*d*) *Influence of synthetic Tannin Agents*: Tests of toxic influence of synthetic tanning agents on bacteria were carried out by the same method as in the case of vegetable tanning agents. For tests of this kind we used the following agents : Kortan SP, Kortan SN_{25}, Kortan QD, Biltan Il.

We noted that the toxic components of those synthetic tanning agents are also substances characterized as tannins. Their effect is instantaneous through a toxic shock so that the quantity of microbes is considerably reduced on instantaneous contact with the tannin and their number decreases during the following hours of testing. The least toxic effect is found with the bleaching

tannin Biltan II. The most toxic are the tannin components of the synthetic tanning agent SN_{25} followed by Kortan SP (Table 7). The tests proved

TABLE 7

Influence of synthetic tanning agents upon microorganisms

Tanning agents	LD_{50} mg/l		
	Staphylococcus aureus	*Bacillus megatherium*	*Psychrophile bacteria* (mixture)
Kortan SN_{25}	0.52	0.70	3.20
Kortan SP	2.30	7.90	11.00
Kortan QD	12.50	21.90	72.00
Biltan II	...	140.00	390.00

that *S. aureus* was most sensitive towards all tested tanning agents while the most resistant was the mixture of Psychrophile bacteria in waste-waters. When comparing, the bactericidal effects of synthetic tanning agents possess an inhibitory effect at far lower concentrations. The active components in synthetic tanning agents are simpler chemical compounds (starting products are compounds of phenol and naphthol) which are obviously more toxic to organisms than the more complex polyphenolic substances found in vegetable tanning agents.

These substances which affect the growth of the microbial population affect also other metabolic processes as could be observed in studying the effects of tanning agents on the respiration of *B. megatherium*. The results characterizing the respiration charges of this microbe caused by the action of Kortans are given in Fig. 5, 6, 7. The diagrams show the fact that all concentrations of the synthetic tanning agents under studying cause respiration changes. Tannins in Kortan SP and SN_{25} (quantities up to 5 mg/l) cause a considerable stimulation of breathing, while the concentration 25 mg/l reduces the consumption of oxygen. Tannins in Kortan QD reduce the oxygen consumption in all the tested concentrations. The increased intensity of breathing is a protective rection of a microorganism and is not caused by the growth stimulation and by assimilation of tannins as substrata, which is evidenced by Fig. 8 characterizing the reproduction of *B. megatherium* in the course of respiration tests.

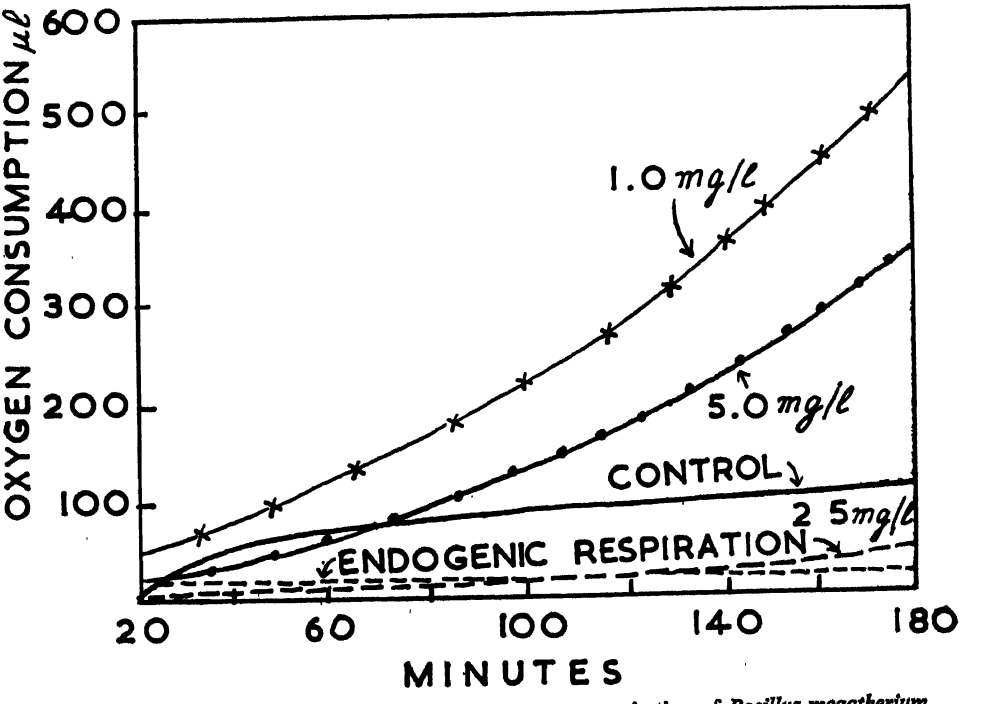

Fig. 5 Influence of Kortan SP tanning agents upon the respiration of *Bacillus megatherium*

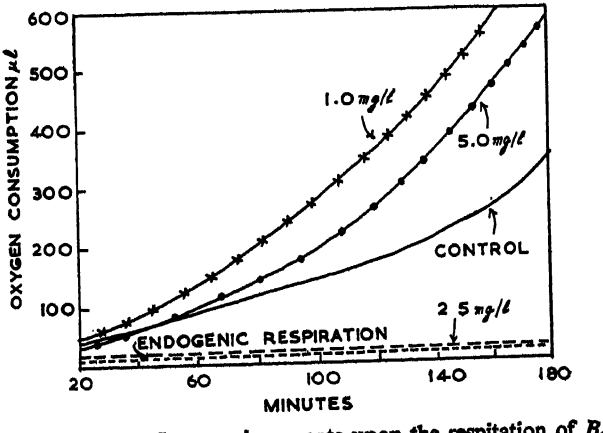

Fig. 6 Influence of Kortan SN as tanning agents upon the respitation of *B. megatherium*

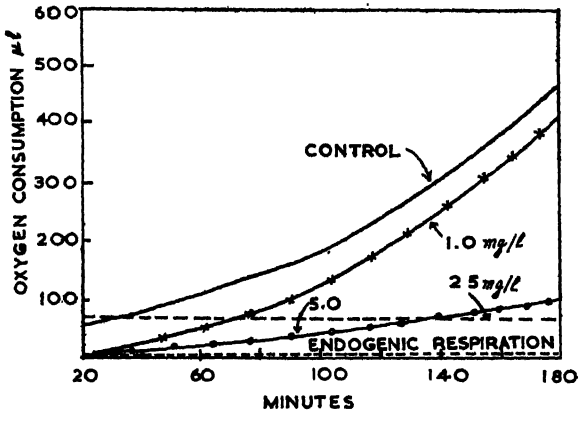

Fig. 7 Influence of Kortan QD tanning agents upon the respiration of *B. megatherium*

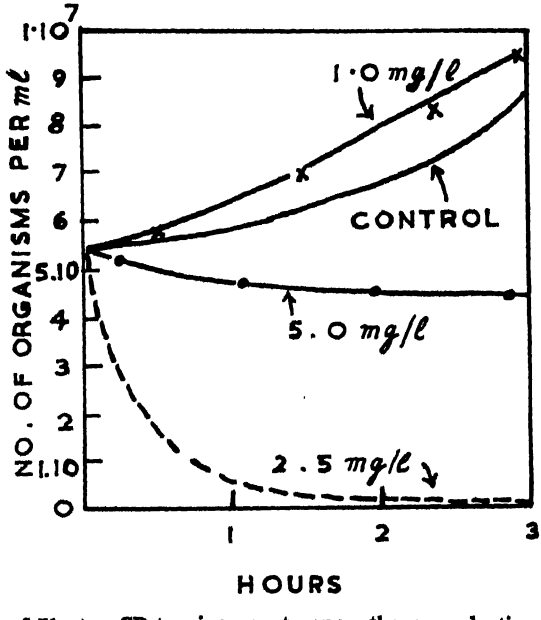

Fig. 8 Influence of Kortan SP tanning agents upon the reproduction of *B. megatherium*

In conclusion, we should add that the identification of the negative effects of tannery chemicals and tanning agents on micro and macroorganisms was a successful solution of biological purification of the tannery waste-waters and this was confirmed by the results obtained.

In the foregoing, the author has dealt with some aspects of microbial problem in the tannery. To solve them, it will be necessary to study and to clearup various casual relations between hide and leather as substratum and between microbes and their metabolic products and to start from this standpoint in solving the questions of controlling and eventually utilizing the microbial processes in the tannery.

Acknowledgements

In conclusion, we wish to thank the Technical Management of the Research Institute for permission to publish these results, and the team of workers in the microbiological laboratory of the Institute for their technical co-operation.

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Influence of pH and Sodium Chloride Concentration on the Development of Aspergillus, Penicillium and Paecilomyces Isolated from Pickled Pelts

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The study of the influence of sodium chloride on the growth of eight species of fungi from pickled pelt has been carried out at different *p*H levels. A 20% concentration of sodium chloride in the basal medium, was lethal to *Penicillium purpurrescens, Paecilomyces varioti, Apsergillus niger, A. flavus, A. oryzae, A. terreus* and *A. ochraceous* at *p*H 2.0. At *p*H 3.0 and above, the increasing concentrations of sodium chloride in the medium affected their growth in different ways. Initial concentrations of NaCl enhanced the growth of *A. niger, A. ochraceous* and *Penicillium purpurrescens* while *Paecilomyces varioti, A. oryzae* and *A. terreus* were sensitive to an increase in sodium chloride concentration in the basal medium, and increasing concentrations produced progressive inhibition of growth.

Sheep and goat skins are exported as pickled pelts by some countries the former being an important export commodity of New Zealand and Australia, while goat pelts are also exported from India. Properly pickled pelts can withstand storage for prolonged periods. However, they are prone to microbial damage, since moulds can develop at low *p*H levels[1]. A number of species of fungi have been reported on Australian sheep pelts[2,6] and ten species were reported from Indian pickled pelts[7].

Acid and salt concentration are two important factors which influence the development of moulds and the quality of the pickled pelt. The presence of sodium chloride in the pickle has a dual role to play, namely it suppresses acid swelling of the pelt and inhibits the growth of microorganisms. Though

concentration of NaCl above 10% retarded the growth of moulds[2,3] it is not uncommon to find germination of mould spores at a salt concentration of 20 to 22%[8]. Enhanced growth with 2 to 4% sodium chloride in the medium was reported with *A. niger*, *A. flavus*, and *Labyrinthula* species[9,10]. Pleass[1] carried out experiments on the pickling of sheep and lamb skins and stated that optimum concentration of sodium chloride and sulphuric acid were 10% and 1% respectively. Moreover, the skins were found to be in good condition for a period of 3 months at temperatures ranging from 0 to 37°C Deficiency of acid and salt in the pickled pelt resulted in mould growth ; while excess of acid and deficiency of salt caused acid damage ; an excess of salt and less of acid produced looseness of structure. Acid hydrolysis led to the yellowing of pickled pelts[11] and later Bowes and Mitton[12] demonstrated, that the acid concentration usually recommended, is dangerous to the pelt if the storage temperature exceeded 30°C[3]. They suggested that more work should be done on fungicides.

The presence of organic acids, acetic acid in particular in pickle with sulphuric acid and sodium chloride prevented mould growth on the pelt[13]. Mould growth was severe if the *p*H of the water extract exceeded 2.5 unless a higher salt concentration inhibited the same[14]. However, the growth was effectively checked by the addition of suitable fungicides [2-5,15-19] and chemicals[22 23].

The wooden containers also contributed to the formation of mould on pickled pelts by absorption of pickle[3]. While, White and Caughley[20] reported that the preferential absorption of salt from free pickle liquor resulted in the mould growth. Damaged casks permitted the infection of pelts by mould spores in the ships holds[21]. It is also dangerous to use wood infected with *Alternaria tenuis* for making casks, as this species grows at low *p*H levels[5]. It has been recently reported that pelts containing higher moisture were more prone to mould attack[12].

The little information that is available on mould growth on pickled skins deals with some occasional determinations of acid and salt tolerance of a few moulds. Detailed study, on the fungicidal and fungistatic action of acid (sulphuric acid) and salt (sodium chloride) appears to be necessary to understand the development of moulds during the storage of pickled skins. Information on the ability of these fungi to develop on media of varying *p*H levels and salt concentrations would throw light on the physiology of these organisms. Hence, study of the initial *p*H and sodium chloride concentrations with particular reference to those obtainable in pickling has been taken up in Laboratory media on fungi isolated from pickled pelts.

Materials and Methods

20 ml of Czapek Dox medium were taken in each of 100 ml Erlenmeyer flasks. The concentrations of sodium chloride in the medium were 0, 5, 10, 15 and 20% (w/v) and the initial *p*H levels were 2.0, 3.0, 4.0, 5.0 and 6.0 respectively. *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. ochraceous*, *A. oryzae*, *Paecilomyces varioti*, *Penicillium purpurrescens* and *P. corylophilum*, isolated from pickled pelts served as test organisms. The *p*H of the media was adjusted using a Beckman *p*H meter with sulphuric acid. The media were sterilized and the inoculum was prepared as follows. To ten day old slant of each fungus species 5 ml of distilled water were added and gently shaken. Spore suspension obtained was decanted aseptically into a sterile test tube, thoroughly mixed and 0.1 ml of this uniform suspension served as inoculum. The cultures were incubated for 10 days at 25 $\pm$ 1°C, and the presence or absence of growth, presence of sporulation and in cases where growth was present a quantitative estimate of growth, were determined. In the case of flasks, where no visible growth was recorded, the respective media were tested for viable spores. This was done by centrifuging the medium and one ml of the sediment was transferred to 25 ml of sterile Czapek medium at *p*H 6.7 under aseptic conditions. The presence of viable spores was indicated by the establishment of growth in the fresh medium which was incubated at 25°C for 15 days.

Results and Discussion

Penicillum purpurrescens (Table 1). This species recorded more mat weight than the control (without salt) at 5% and 10% salt concentrations at *p*H levels 3.0-6.0. An appreciable drop in the growth was, however, observed at 15% sodium chloride concentration and growth was negligible at 20% level. At *p*H 2.0 this fungus thrived well in the control, and there was a drastic reduction in the mat weight in the presence of salt. The growth was inhibited at 15% and 20% salt concentrations, the latter being lethal to the spores (Fig. 1).

P. corylophilum (Table 1). The basal medium at *p*H 3.0 to 5.0 supported the growth of this fungus better than that with salt. A decline in the mat weight was noticed with increasing concentrations of salt, this being more marked above 10% salt levels than that in the control. High acidity (*p*H 2.0) was unfavourable for its growth, the mat weight being poor upto 5%. At higher concentrations of salt its growth was totally inhibited (Fig. 1).

TABLE 1

Mat weight at different pH levels and sodium chloride concentrations in Czapek Dox medium in mg

Name of the organism	Initial *p*H of the Medium	Percentage concentration of sodium chloride									
		Nil		5		10		15		20	
		Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H
Penicillium purpurrescens	2.0	101.4	2.4	32.4	3.5	9.0	3.5	Nil	2.0	Nil	2.0
	3.0	231.0	3.6	237.0	5.4	258.0	4.5	20.6	3.4	1.0	3.0
	4.0	180.0	3.9	189.0	5.9	219.0	5.5	23.2	4.2	3.0	4.0
	5.0	165.0	3.7	193.0	5.5	229.0	5.5	24.8	4.9	7.0	5.0
	6.0	150.0	3.7	192.0	5.5	217.0	5.7	22.0	5.9	5.0	6.0
P. corylophilum	2.0	2.0	2.1	2.0	2.0	Nil	2.0	Nil	2.0	Nil	2.0
	3.0	261.0	5.5	200.4	6.8	185.0	5.8	104.8	4.9	5.4	3.8
	4.0	248.0	5.5	221.0	7.8	206.0	6.0	69.0	5.1	8.6	4.7
	5.0	331.0	7.6	231.4	7.7	221.6	6.9	40.2	5.0	5.0	5.0
	6.0	170.1	7.9	218.0	7.3	214.0	6.9	22.0	6.3	8.0	6.2

Paecilomyces varioti (Table 2.) As in the case of *Penicillium corylophilum* the *p*H level of 2.0 was not conducive to the growth of *Paecilomyces varioti.* 10% salt concentration was inhibitory to this fungus at this *p*H while at 15% and 20% concentrations the spores were killed. This species thrived better at *p*H levels 3.0-6.0 in the absence of salt. With increase in salt content even at these *p*H levels the growth progressively decreased, becoming almost negligible at 20% (Fig. 1).

Aspergillus terreus (Table 2). At *p*H 2.0 no visible growth was recorded in all cases, including control without salt. 20% salt concentration was lethal to the spores at *p*H 2.0 and inhibitive at *p*H 3.0. The spores, however, remained viable up to 15% concentration at *p*H 2.0 and 20% at *p*H 3.0. At *p*H values 3.0 to 6.0, this species was susceptible to NaCl concentrations and a sudden drop in the mat weight was noticed, above 5% level (Fig. 1).

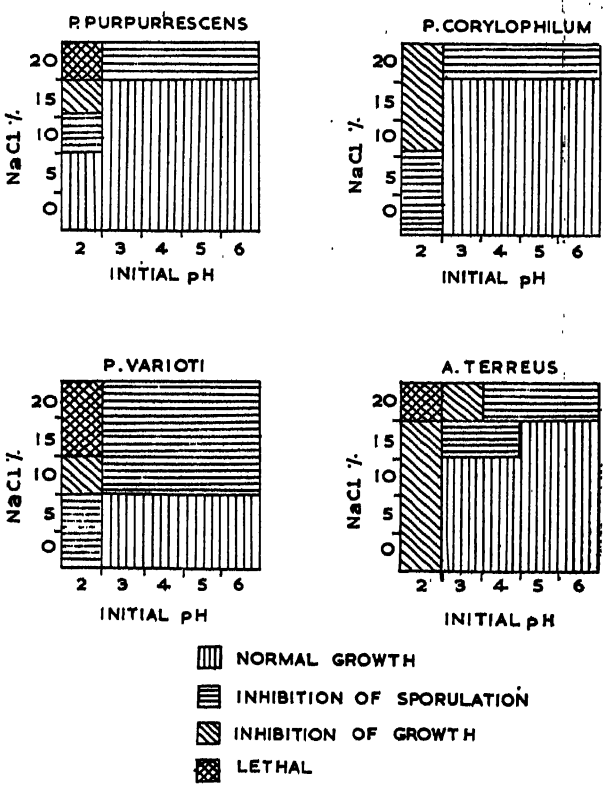

Fig. 1 *Effect of sodium chloride concentrations on the growth of fungi at different pH levels*

TABLE 2

Mat weight at different pH levels and sodium chloride concentrations in Czapek Dox medium in mg

Name of the organism	Initial pH of the medium	percentage concentration of sodium chloride									
		Nil		5		10		15		20	
		Mat weight	Final *p*H	Mat wegith	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H
Paecilomyces varioti	2.0	20.6	2.1	7.0	2.2	Nil	2.0	Nil	2.0	Nil	2.0
	3.0	184.6	3.1	104.0	3.1	31.4	3.0	23.0	3.0	1.0	3.0
	4.0	187.3	3.5	155.5	3.5	58.4	3.7	33.0	3.9	2.4	3.9
	5.0	146.0	3.5	78.6	3.9	33.6	3.9	14.0	4.7	2.5	4.8
	6.0	136.0	3.7	56.6	4.7	8.0	5.3	6.2	5.8	1.0	5.9
Aspergillus terreus	2.0	Nil	2.1	Nil	2.0	Nil	2.0	Nil	2.0	Nil	2.0
	3.0	308.4	8.5	229.4	5.5	85.0	5.5	8.6	4.0	Nil	3.2
	4.0	324.4	8.4	244.4	6.6	80.0	5.9	47.6	5.6	9.4	4.8
	5.0	280.2	8.2	193.0	5.7	72.7	5.5	52.8	5.3	8.6	5.0
	6.0	144.8	7.2	31.6	5.9	27.6	5.9	19.0	5.9	4.0	5.9

A. niger (Table 3). The presence of salt in the basal medium upto 10% increased the mat weight at *p*H levels 3.0 to 6.0 and at 15% concentration and above sudden drop in mat weight was observed. As contrasted against good growth at *p*H 2.0 in the salt free control medium, a marked drop in mat weight was observed at 5% and 10% concentrations followed by complete inhibition at 15% concentration. At *p*H 2.0 viable spores could not be detected at 20% salt level (Fig. 2).

A. oryzae (Table 3). Growth was inhibited at *p*H 2.0 upto 10% salt content and above this the spores lost viability. 5% sodium chloride in the medium resulted in more mat weight, than in the controls at *p*H level 3.0 to 6.0. A further rise in sodium chloride content was followed by a significant decline in mat weight. With 20% sodium chloride growth was negligible at *p*H 3.0 to 6.0 (Fig. 2).

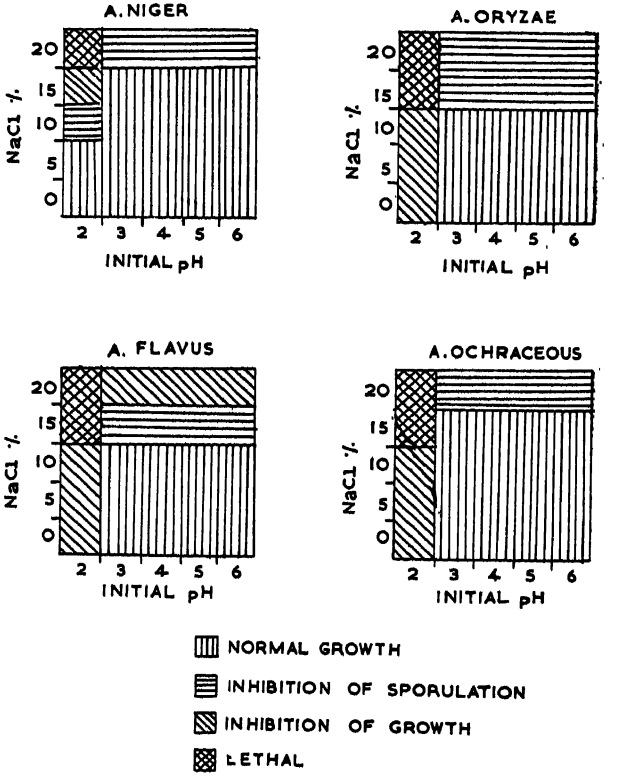

Fig. 2 *Effect of sodium chloride concentrations on the growth of fungi at different pH levels*

TABLE 3

Mat weight at different pH levels and sodium chloride concentrations in Czapek Dox medium in mg.

Name of the organism	Initial pH of the medium	Percentage concentration of sodium chloride									
		Nil		5		10		15		20	
		Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H
A. niger	2.0	140.0	2.4	32.5	2.1	3.4	2.0	Nil	2.0	Nil	2.0
	3.0	135.7	2.5	196.6	3.3	153.0	3.6	72.0	2.6	4.3	3.0
	4.0	114.3	2.5	174.3	3.2	151.0	3.9	60.0	3.9	4.4	3.8
	5.0	112.2	2.4	114.3	3.2	115.0	4.0	68.0	4.2	4.0	5.0
	6.0	104.2	2.4	120.0	3.2	100.0	4.0	50.0	4.8	6.0	5.9
A. oryzae	2.0	Nil	2.1	Nil	1.9	Nil	1.9	Nil	2.0	Nil	2.0
	3.0	147.8	5.4	177.4	6.9	132.0	5.9	131.0	3.7	5.0	2.9
	4.0	160.2	5.4	204.0	7.2	96.0	5.5	39.4	5.0	2.4	3.0
	5.0	153.5	6.0	188.7	5.2	47.0	5.2	36.4	5.1	1.4	5.0
	6.0	107.0	6.9	141.6	6.2	47.2	6.3	1.2	5.8	1.0	5.9

A. flavus (Table 4). The behaviour of this fungus was similar to that of *A. oryzae at* *p*H 2.0. An increase in mat weight was recorded at the salt concentration of 5% at *p*H levels 3.0-6.0. Further increase in salt concentration beyond 5% resulted in decreased mat weight and the growth was inhibited at 20% (Fig. 2).

A. ochraceous (Table 4). As in the previous two species, the growth of this species was inhibited at *p*H 2.0. At *p*H 3.0 to 6.0 this fungus was found to tolerate even 15% salt in the basal medium as seen from the mat weight. It is noteworthy, that maximum growth was recorded at 15% level. At *p*H 2.0, however, the growth was inhibited. At this *p*H, in salt concentrations upto 10%, the spores remained viable, while at 15% and 20% levels they were killed. (Fig. 2).

The influence of *p*H and salt concentrations in the physiological development of these moulds are also depicted in separate diagrams (Figs. 1 and 2). From these, it is clear that, at higher concentrations of sodium chloride, reduction in growth was accompanied by suppression of spore formation. The lethal and inhibitory concentrations are also shown.

The present studies with *A. niger*, *A. terreus*, *A. oryzae*, *A. ochraceous* and *P. purpurrescens*, *P. corylophilum* and *Paecilomyces* indicated that growth was possible even at 20% salt concentration at *p*H 3.0 to 6.0. This corroborates the earlier findings of Blank[2] that some species are capable of growth at 18% salt concentration on solid media at *p*H 4.3. A 20% concentration of salt seems to suppress the growth of all the species except *A. ochraceous*. At other salt levels the inhibitory effect seems to depend on the species and *p*H of the medium. This clearly brings out the importance of the *p*H as well as salt in preventing mould growth. It is also significant that the spores are destroyed at 20% sodium chloride concentration at *p*H 2.0 excepting *P. corylophilum* (vide Table 1, Fig. 1). The findings of Doelger (cited by Clank[2]) that the tolerance of moulds to sodium chloride remained constant at *p*H levels 3.0 to 7.0 does not seem to be supported by the present results as lower salt concentrations actually enhanced the growth in some species and higher concentration inhibited the same at *p*H levels 3.0 and 4.0. The present results, however, cannot be compared with those of Stuart and Frey[3] as their study was made with *Alternaria* and *Penicillium* species, where it has been reported that 12% salt concentration does suppress the growth even at *p*H 2.4.

The results also bring out one important point with reference to pickling. The usually recommended concentration of salt for pickling is 10%[1]. Though

TABLE 4

Mat weight at different pH levels and sodium chloride concentrations in Czapek Dox medium in mg

Name of the organism	Initial *p*H of the medium	Percentage concentration of sodium chloride									
		Nil		5		10		15		20	
		Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H	Mat weight	Final *p*H
A.ochraceous	2.0	Nil	2.0	Nil	2.0	Nil	2.0	Nil	2.0	Nil	2.0
	3.0	70.0	3.1	195.0	8.5	326.5	8.6	360.2	7.5	13.8	3.1
	4.0	260.0	8.1	258.6	8.7	346.0	8.6	385.8	8.6	130.8	5.5
	5.0	247.0	8.1	253.8	8.7	342.0	8.6	361.4	8.1	157.0	5.7
	6.0	240.0	7.8	254.4	8.8	346.0	8.6	316.4	8.1	133.2	6.8
A. flavus	2.0	Nil	2.0	Nil	2.0	Nil	2.0	Nil	2.0	Nil	2.0
	3·0	98.7	6.9	96·0	6,6	65.0	5.8	9.0	4.3	Nil	2.9
	4.0	92.6	7.5	110.0	6.9	53.0	5.5	34.0	4.7	Nil	4.1
	5.0	80.8	7.3	135.0	6.5	43.6	5.7	29.0	5.4	Nil	5.0
	6.0	80.2	7.2	75.0	6.4	37.0	6.5	23.8	6.2	Nil	5.9

at *p*H 2.0, this salt concentration is effective in preventing mould growth, it fails to do so at *p*H 3.0 in Czapek Dox medium (Table 5). As the pH of pickled

TABLE 5

Optimum, inhibiting and lethal sodium chloride concentrations and *p*H levels of different species of *Aspergillus* and *Penicillium*

Name of the species	Inhibiting NaCl concentration at *p*H 2	Lethal NaCl concentration at *p*H 2	Optimum NaCl concentration and initial *p*H
Penicillium purpurrescens	15%	20%	5 & 10% at *p*H 3.0
P. corylophilum	10—20%	...	5% at *p*H 6.0
Paecilomyces varioti	10%	15 & 20%	5% at *p*H 4.0
Aspergillust erreus	0—15%	20%	5% at *p*H 3.0 & 4.0
A. niger	15%	20%	5% at *p*H 3.0
A. flavus	0—10%	15% & 20%	5% at *p*H 5.0
A. oryzae	0—10%	15% & 20%	5% at *p*H 4.0 & 5.0
A.ochraceous	0—10%	15% & 20%	15% at *p*H 3.0 to 5.0 and 10% at *p*H 4.0

pelts is likely to increase under conditions of storage, a salt concentration that can inhibit growth at pH 3.0 appears preferable. This is more significantly brought out by the fact that spores of certain moulds, *Paecilomyces varioti, Aspergillus terreus, A. flavus, A. oryzae* and *A. ochraceous* which remain viable at *p*H 2.0 at this salt level become sources of inoculum for growth at higher *p*H 3.0 (Figs. 1 and 2).

The risk of depending upon sodium chloride concentration upto 10% for inhibiting growth is brought out, by the behaviour of *A. niger, P. purpurrescens* and *A. ochraceous*. These recorded higher mat weights at initial *p*H levels of 3.0 and above in the presence of 5-10% salt. This clearly brings out that while at *p*H 2.0 this salt concentration may inhibit growth, it actually

accelerates growth at higher *p*H levels. In the case of *A. flavus* such a phenomenon was observed upto 5% salt at pH 5.0 while with *A. oryzae*, it was observed at pH 3.0 to 6.0. However, *A. terreus*, *P. varioti* and *P.corylophilum* appeared to be susceptible to salt concentrations, progressive reduction in growth being observed with increase in salt concentration. An interesting observation was observed with *P. corylophilum*, where a peculiarity was noted *i.e.*, at *p*H 5 and 6 the growth was enhanced at 5 to 10% salt which at 15% level produced an inversion in the growth curve. This indicates that a stimulatory effect was followed by one of suppression. Thus sodium chloride suppresses growth at *p*H 2.0 but at *p*H 3.0 such growth is considerably enhanced by lower concentrations of sodium chloride. The presence of sodium chloride in the medium at 5% level was responsible for shifting the *p*H optima by one unit (e.g. *P. corylophilum*, *A. terreus*, *A. niger*, *A. flavus*, *A. oryzae*). However, at this optimum pH level 5% salt concentration enhanced this growth. *A. ochraceous*, on the other hand, showed the shifting of the optimum *p*H level to a lower unit (from a *p*H level of 4.0—6.0 to 3.0—5.0) in the presence of even 15% sodium chloride. Perhaps the salt at stimulatory levels increase the uptake of nutrients especially nitrogen.

Viability of spores at pH 2.0 at salt concentrations upto 20% has been demonstrated for the first time. This may perhaps explain the development of moulds on pickled pelts in the absence of extraneous infection. A perusal of table 5 reveals that spores of *A. flavus*, *A. oryzae*, *P. varioti* and *A. ochraceous* remained vaible upto 10% salt and those of *P. purpurrescens*, *A. niger* and *A. terreus* upto 15% salt. The viability of the spores of *P. corylophilum* was maintained even at 20% salt. Experiments on soluble constituents of pickled pelt at 10% salt have since shown that the spores remained viable even at the end of a month.

Results presented with regard to the influence of acidity, *vis-a-vis* sodium chloride concentrations used in pickling of skins, had indicated a few facts which can be used as pointers to any further formulation of improved pickling media. The *modus operandi* of these two factors in preventing mould growth appeared to be essentially complementary to each other. Acidity increased the effect of salt by the virtue of its influence on ionisation, and salt increased the influence of acidity by supplying ions like (Cl) that could compete or even prevent the uptakes of essential ions like nitrate (NO_3). The fact that there was growth at low *p*H in the absence of sodium chloride seemed to suggest that a low *p*H in itself was not detremental. As salt concentrations upto 10% or 15% supported growth in the absence of inhibitory *p*H levels, it suggests that neither of them alone is capable of bringing about a complete inhibition of mould activity except at high concentrations.

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Study on the Red Colouration on Chrome Blue

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Aspergillus, Penicillium and *Paecilomyces* species were isolated from red pigmented wet blue chrome leather from Calcutta, Kanpur and Madras origin. *Penicillium purpurogenum* (Stoll) was found to be mainly responsible for the development of red colouration. Some of the characteristics of the pigment developed by the above mould have been described and a method for the extraction of pigment from the aqueous medium using iso-amyl alcohol is suggested.

Appearance of red coloured spots and patches on wet chrome blue is a phenomenon frequently observed in the leather trade and is an important problem facing Indian Leather exporter as it lowers its quality considerably. Wet chrome blue is highly acidic in nature, with a *p*H value varying between 3.5 and 4.0 and as such the possibility of bacterial development may be ruled out. However, mould growth may take place. Chrome leather in itself does not constitute a good medium for mould growth and its acidic nature and absence of any noticeable mould growth at the early stages of pigmentation has created considerable doubt about its origin. The presence of small amounts of sugar or molasses (used for reduction of chrome liquor) for fatty matter in the leather was conducive to mould growth [1-3]. Mitton and Turner[4] isolated for the first time *Penicillium islandicum* and some *Aspergilli, Penicillia* and *Paecilomyces*, from pigmented leather. Orlita[5] isolated *P. aculeatum* which secreted extracellular pigment on chrome leather. This pigment was red in acid and yellow in alkaline medium. Sen[2] opined that *P. rubrum* (Stoll), a member of the same family as that of *P. islandicum* reported earlier[4], was the main source of pigmentation on chrome blue. Both the species, however, belong to Biverticillate series, while Bartramth and Reisse[6] suggested that the species causing such

pigmentation belongs to *Monoverticillate* series. The use of a number of fungicides for their prevention was suggested by Sen[8] and some factors infl encing pigment production and certain characteristics of the pigment were reported[7,8]. Literature cited indicate that their studies have been mostly sporadic and that a thorough investigation on red colouration of chrome blue was necessary. Contrary to the popular belief, the occurrence of such colouration in the absence of any sugar or molasses has been brought to the notice of the author. Discolouration of consignments, treated with fungicides prior to shipment has also beenr eported. A thorough study of this important problem, was, therefore, warranted.

In the present investigation attempts have been made to isolate and identify the species of fungi associated with pigmentation, and to study the properties of the pigment produced.

Materials and Methods

Wet chrome (blue) leather with red patches and spots were collected from tanneries in Calcutta, Kanpur and Madras. The coloured portions were cut into small pieces and shaken for two hours in sterile distilled water and the shake liquors were used for the isolation of moulds. The methods outlined by Commenwealth Mycological Institute[9] were followed for the purpose of isolation and identification. The strains obtanied were used in studies growth and pigmentation on the following media (a) Czapek-Dox agar, (b) Malt extract agar, (c) Sabouraud's agar, (d) Chrome leather extract glucose agar.

Chrome leather extract glucose agar medium was prepared by boiling 5 g wet chrome leather (tanned with sulphate reduced chrome liquor) cut into small pieces (2.5 $\times$ 2.5mm), in distilled water for 15min filtered through fine linen and made upto 100 ml. After the addition of 2 g of glucose and 3.5 g agar the pour plates were prepared.

Chrome tanned hide powder glucose agar medium; 5 g chrome tanned hide powder (tanned with sulphate reduced liquor) was suspended in 100 ml distilled water and to this 2 g of glucose and 3.5 g agar were added.

Growth and pigment developed by the isolates in the above media were studied at 28$\pm$2°C. The colour on the reverse was compared using the "Dictionary of Color"[14]. The observations of 15 day old cultures are presented in Table 1.

The nature of mould attack and subsequent colouration of leather were also studied histologically. For this purpose chrome leather (tanned with sulphate reduced liuqor) was soaked for 2 hr in 2% glucose solution, sprayed with a suspension of mould spores, and stored in a humid chamber at room temperature (28±2°C). Observations were made periodically on samples from the treated leather and were mainly confined to the attack on grain side as the pigmentation of the grain was considered more injurious than that of the flesh side.

In the subsequent studies liquid media were used. The preliminary experiments indicated that Czapek dox solution not only gave good growth but also resulted in a good amount of pigment production which diffused in the medium. Chrome-tanned leather extract glucose and chrome-tanned hide powder glucose media gave only submerged growth in Czapek dox medium. Normal growth and pigmentation were, however, restored. Hence, Czapek dox solution was chosen for the subsequent studies.

Results and Discussion

Ten species of moulds were isolated and indentified. They were *Aspergillus niger*—Van Tieghem, *A. terreus*—Thom, *A. flavus*—Link, *A. fumigatus*—Fresenius, *Paecilomyces varioti*—Bainier, *Penicillium janthenellium*—Biourge *P. chrysogenum* series, *P. brefeldianum*—Dodge, *P. purpurogenum*—Stoll and *P. varians*—Smith.

Of the different species isolated, *P. purpurogenum* and *P. varians* produced red pigment in Czapek dox agar medium. *P. purpurogenum* was common in all but for a single occurrence of *P. varians* in association with *P. purpurogenum*. The later was mainly responsible for the pigmentation of chrome blue. This finding, however, differs from those of the early workers. Cordon[10] reported the presence of the first five species of the present study on leather whereas the existence of *Paecilomyces varioti* was indicated by Mitton and Turner[4]. The remaining four species were reported for the first time in the present study. The reports of the Commonwealth mycological Inst., Surrey on the identification of *P. purpurogenum* Stoll are given below :

"Isolates I-IV are all the strains of *P. purpurogenum Stoll* and there is considerable variation in cultural characteristics."

TABLE 1

Growth of mould and colour developed (After 15 days)

Species of mould	(1) Czapek Dox		(2) Malt ext. Glucose		(3) Sabouraud's		(4) Cr. Leathe Ext. Glucose		(5) Cr. Tan Hide Powder Glucose	
	Growth	Colour on the reverse	Growth	Colour on the reverse	Growth	Colour on the reverse	Growth	Colour on the reverse	Growth	Colour on the reverse
A. niger	+++	12D1	+++	14H5	++++	15L4 (Olivegreen)	+	—	+	—
A. terreus	++	13K7 (Isabella)	+++	13K5	+++	13K8 (Centenial Brown)	+	—	+	—
A. flavus	++++	21B1 (Quaker-grey)	+++	21C2 (Teagreen)!	++++	12KG (Burnt yellow stone)	+	—	No Growth	—
A. fumigatus	+++	2011	+++	20H1	++++	13L1	+	—	—	—

Paecilomyces varioti	++++	13E1	++++	12E2 (Sallow)	++++	1312	++	12C2	No growth	—
Penicillium chryosgenum	++++	12L7 (Barnished gold)	++++	11J4 (Mustard)	++++	11J4 (Mustard)	++	—	—	—
P. janthenellium	+++	10H3	+++	20G1	++++	13K5	++	—	+	—
P. brefeldianum	+++	11L7 (Yellow Ochre)	+++	12J2 (Absinthe yellow)	+++	18F2	++	—	+	
P. purpurogenum	+++	5K9 (Rose vale)	++	5G11	+++	3L11 (Blood red)	+	11A7 (Onion skinpink)	+	5B2
P. varians	++++	5J8	+++	3E12 (Burnt orange)	+++	15A12 (Burnt umber)	++	11A7 (Onion skinpink)	+	5C9 (Rose blood)

—Very scanty ; + Poor ; ++ Medium ; +++ Heavy ; ++++ Very heavy ; —No colour.

The fungal flora of wet chrome blue obtained from different parts of India varied considerably and list of species isolated is given below :—

CALCUTTA : *A. niger, A. terreus, A. flavus, A. fumigatus, Paecilomyces varioti, P. chrysogenum, P. brefeldianum, P. purpurogenum.*

MADRAS : *A. niger, P. chrysogenum, P. janthenellium, P. varians, P. purpurogenum.*

KANPUR : *A. niger, A. flavus, A. terreus, Paecilomyces varioti, P. brefeldianum, P. janthenellium, P. chrysogenum, P. purpurogenum.*

The above variation may be attributed to the environmental factors and the manufacturing processes followed in different tanneries. It is observed that *A. niger, P. chrysogeum* and *P. purpurogenum* were common in all the samples studied. From Table 1, it is noticed that there was good growth in all the media excepting media 4 and 5, where the growth of most of the isolates was either partially or completely inhibited.

P. purpurogenum produced pigment on varying shades from purple to pink and which diffused into the media. Though the growth of this strain was much retarded, it produced fairly good amount of pigment in the chrome leather extract and chrome tanned hide powder glucose media. The pigment exhibited a great affinity for chrome tanned fibres which were readily stained pink in medium No. 5. *P. varians* also developed similar colouration in media 1, 4 and 5 but was found less diffusing. The above observations explain the absence of any visible mould growth on leather at the initial stages of red colouration.

The development of red colouration on leather took place both on the grain and the flesh sides, but it was more frequently noticed on the grain side. Pigmentation on the grain side is considered more harmful because in such cases it is rather difficult to impart a uniform finish to the leather. The nature of penetration of mould into plant bodies have been studied by a number of investigators [11,12,13] and the nature of infection described to have been occurred in stages depending upon the structure of the host and the species of mould. But no such information was, however, available in respect of leather as the host material. Earlier studies by the author and Ghouse (under publication) revealed that mould attack and subsequent pigmentation on the grain of leaher took place in stages and the same has been briefly summarised below :

(i) Mould spore accumulation at the mouth of the hair follicles and development of mucilaginous membrane, (ii) germination of spore and development of mycellium net work inside the follicle, (iii) production and diffusion

of pigment, (iv) appearance of fertile hyphae at the mouth of the hair follicle and (v) spreading up of the mycelia on the grain surface. The above findings are not in close agreement with those of the early workers but there exist some similarities between them at some stages of mould attack. This variation is probably due to many reasons. Firstly, the earlier workers confined their studies on the penetration of moulds on plant bodies which are alive whereas in the present study the host is an inert material. Secondly, leather is in highly acidic condition which may not be a very favourable substrate for mould growth. Lastly, the physical structure of leather is totally different from that of the substrates employed by the earlier workers, and, hence, it is, but, natural to have some variations.

Some properties of the pigment : The pigment produced by the mould readily diffused into the medium and the colour of the solution, ranged from purple to pink. Spectrophotometric study indicated a shift in absorption maxima with the period of incubation and the *p*H of the solution. The absorpti on maxima, however, remained in the range from 465 to 485 mμ.

Incubation period	Absorption maxima at different pH levels		
days	*p*H 3.5	*p*H 5.3	*p*H 9.0
7	475 mμ	485 mμ	485 mμ
14	475 mμ	480 mμ	485 mμ
21	465 mμ	465 mμ	470 mμ
28	465 mμ	465 mμ	465 mμ

The above results suggest that the pigment possessed some indicator effect with the *p*H of the solution.

The pigment developed in the medium was completely absorbed by activated charcoal and attempts of its recovery met with no success. The pigment was also precipitated by lead acetate solution from which it was possible to recover the pigment in much purer from by chemical methods and it showed a great affinity for chrome tanned hide fibre. The pigment was extractable from the solution by a number of solvents. In the present study isoamyl alchol was employed for extraction and the method is as follows :

50 ml of the coloured medium after removing th. e mycelial pad was centrifuged for one hr at 200 rpm. The solution was taken in a separating funnel and s ·' gourousiy with 20 ml petrol ether and allowed to stand. Petrol ether layer which was almost colourless was separated. The aqueous layer was extracted 3-4 times with 5 ml portions of amyl alcohol which removed most of the pigment. The residual portion of the pigment was, however,

extracted with further treatments of amyl alcohol after adding 2-3 drops of dilute hydrochloric acid. Amyl alcohol extracts were combined and washed with 25 ml distilled water and allowed to stand for 24 hr. The extract was then centrifuged for 2 hr at 2000 rpm to remove all the suspended particles and then allowed to evaporate at 65°C for 48 hr and finally dried over anhydrous sodium sulphate until the weight was constant.

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Influence of Tannin on the Free Amino Acids Set Up of Some Fungal Hyphae

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Free amino acids present in the mycelium of *Aspergillus niger*, *A. flavus* and *A. fumigatus*, grown on Czapek's solution containing various tannins were studied. Sixteen different free amino acids were found collectively in the mycelium of the above mentioned three species of *Aspergillus*. The addition of tannin was found to increase the quantity of free amino acids in *A. niger* and *A. flavus* and also to produce some new amino acids in *A. niger*.

It is known that fungal growth on vegetable tan liquors is not desirable as these fungi can destroy the tanning properties of liquors some times at a high rate.Most of the earlier workers have observed only the tannin destruction in the liquor by the mould. But it has not been recorded whether the mere presence of these fungi, apart from the metabolism during growth will affect the tanning process as such especially in view of the fact that free amino acids are present in the fungal hyphae[1-3]. Moeller[4] pointed out that in the presence of amino acids in the tan liquor the absorption of tannin by hide powder was decreased. By adding certain amino acids to hide powder-tannin system, he found a decrease in the fixation of tannin to hide powder. So it was thought desirable to find out the occurrence of free amino acids in the mycelium of three species of *Aspergillus* commonly found in tan liquors.

Materials and Methods

Czapek's medium with the following composition was taken as the basal medium. $NaNO_3$—2.00 g, K_2HPO_4—1.00 g, $MgSO_47H_2O$—0.5 g., KCl—0.5 g, $FeSO_47H_2O$—0.01 g, Sucrose 30 g, distilled water 1000 ml. *Aspergillus niger*, *A. flavus* and *A. fumigatus* were employed as test organisms.

The inoculum was prepared as follows. 5 days old cultures on Czapek's Dox agar medium of the respective species were taken and 5 ml of the sterile distilled water were added to it. The test tubes were vigorously rolled for 5 minutes to get uniform suspension of spores and 0.2 ml of these suspensions were used as inocula.

Avaram, myrab and babul tannins were taken for the present study and were prepared by extracting the respective tanning materials with methanol. 1% of the respective tannins was added to the basal medium. The sterilisation was done in a Barnstead Autoclave at 15 psi for 15 min.

250 ml portions of the basal medium were taken and 2.5 g of each of avaram, myrab and babul tannins were added separately to it. One aliquot of 250 ml of basal medium served as control with no tannin. The *p*H of the medium was adjusted to 6.2 in all the cases. 50 ml portions of the basal medium containing 1% of the respective tannins were taken in 250 ml conical flasks and sterilised along with the control as mentioned earlier. Each set was inoculated with the three different species of *Aspergillus* taken for study. They were incubated for 15 days at 30°C before estimating the free amino acids in the fungal hyphae.

Mycelial pads were harvested and washed with distilled water. The free amino acids were then extracted in a blender with 25 ml of 85% alcohol at room temperature[3]. 100 λ of the extract was taken for spotting. Chromatographic analysis was carried out using the two dimensional chromatographic technique of Levy and Chung[5]. Separate control chromatograms were run in each case with known amino acids for comparing and identifying them in the experimental chromatogram.

Results and Discussion

Results obtained with the moulds tested with different tannins in the basal medium are given below. The free amino acid set up in the mycelium of *A. niger* is presented in Table 1.

TABLE 1

Free amino acid set up in the mycelium of *A. niger*

Control wih no tannin	Medium with avaram tannin	Medium with myrab tannin	Medium with babul tannin
Cysteic acid	Cysteic acid	Cysteic acid	Cysteic acid
Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
Glutamic acid	Glutamic acid	Glutamic acid	Glutamic acid
Serine	Serine	Serine	Serine
Asparagine	Asparagine	Asparagine	Asparagine
Glycine	Glycine	Glycine	Glycine
Threonine	Threonine	Threonine	Threonine
Glutamine	Glutamine	Glutamine	Glutamine
Lysine	Lysine	Lysine	Lysine
Histidine	Histidine	Histidine	Histidine
Arginine	Arginine	Arginine	Arginine
Alanine	Alanine	Alanine	Alanine
	Leucine	Leucine	Leucine
	Isoleucine	Isoleucine	Isoleucine

The area and intensity of the amino acid spots obtained with the tannin medium were considerably larger when compared with the control. Most of the amino acids appeared as compact spots except histidine and arginine which tended to trail. Further, two additional spots were obtained with tannin medium. There was no difference in the free amino acid set up of *A. niger* with different tannins. From the size and intensity of the spot it can be said that *A. niger* is able to synthesise more free amino acids in the presence of nitrate as nitrogen source and sugar and tannin as carbon source.

TABLE 2

Free amino acids present in the fungal hyphae of *A. flavus*.

Control without tannin	Medium with avaram	Medium with myrab	Medium with babul
Cysteic acid	Cysteic acid	Cysteic acid	Cysteic acid
Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
Glutamic acid	Glutamic acid	Glutamic acid	Glumatic acid
Serine	Serine	Serine	Serine
Asparagine	Asparagine	Asparagine	Asparagine
Glycine	Glycine	Glycine	Glycine
Threonine	Threonine	Threonine	Threonine
Glutamine	Glutamine	Glutamine	Glutamine
Alanine	Alanine	Alanine	Alanine

The area and intensity of the amino acid spots obtained with the control were comparatively smaller when compared with those of other chromatograms. From the above observations (Table 2), it can be inferred that *A. flavus* is able to increase the quantity of free amino acids in the mycelium when tannin is added to the basal medium.

In the case of *A. fumigatus* there was no difference in the size, intensity and number of spots between the control and experimental with the exception that methionine and/or valine were obtained.

It is known that free amino acids combine with tannins[4]. This interaction naturally will result in reducing the effective tannin content in the liquors. In the present investigation production of larger amounts of free amino acids by *A. niger* and *A. flavus* was found when they were grown in presence of tannin in the basal medium. Further in the case of *A. niger*, two additional amino acids leucine and isoleucine were found when grown in the test media.

Compounds containing nitrogen and phosphorous are always present in living cells and the presence of these two elements in vegetable tanning materials has also been reported. The percentages of nitrogen and phosphorous in various tanning materials were reported by Philips[6] and he found that these tannin materials from fruits or seeds were relatively richer in both nitrogen and phosphorous than the tanning materials from barks or woods. The presence of upto 0.5% nitrogen and 0 2% phosphorous was found in myrobalan extracts. Hathway[7] reported the presence of nearly 18 amino acids in myrobalan extracts. The range of amino acids found after the removal of polyphenols and tannins from 80% (v/v) aqueous methanolic extract of myrobalans was found to be typical of those detected in plant extracts in general. In addition to amino acids, small quantities of phosphoric acid and ester phosphate were also found by Hathway[7] in myrobalan extract. It was reported that some of the constituents which contain nitrogen and phosphorous can be removed from a myrobalan liquor by extraction with ethyl acetate[6]. Since free amino acids or inorganic phosphates are not extracted by ethyl acetate it is evident that myrobalan contains other nitrogenous constituents besides the amino acids. The presence of organic compounds containing nitrogen and phosphorous in the various tanning materials may possibly afford an explanation for the finding that more free amino acids are present in the fungal hyphae grown in the Czapek's medium in the presence of added vegetable tannins.

It is well known that nitrogen and phosphorous are essential for the growth of moulds and yeasts. Tan liquors from myrobalans, alagarobilla and dividivi which contain more nitrogen and phosphorous were reported to undergo fermentation and support mould growth very readily whereas in tan liquors of low phosphorous content obtained from quebracho, chestnut and mimosa, fermentation and mould growth was found to be almost absent[6]. It is possible, therefore, that the presence of compounds containing these two elements in the vegetable tannins used in the present study might have facilitated the growth of the fungi and thus produced more of the free intercellular amino acids in the fungal hyphae. In the present study all the cultures were grown on liquid Czapek's medium with nitrate as the only nitrogen source and sucrose as the carbon source. The fully developed mycelial pads were separated from the medium and were washed with distilled water and then only the free amino acids were extracted by blending with 85% alcohol. The free amino acids present in the vegetable tannins as such cannot contribute to the increased formation of amino acids because these free amino acids will be utilised by the fungi for their growth. The nitrogen of the amino acids can be utilised by fungi is also evident from the work of Close[3]. Most of the fungi which he examined were grown on a liquid medium in which asparagine was the nitrogen source and he had actually observed that a large number of amino acids were present in the fungal hyphae grown in this medium. That the mycelial pads are not contaminated with any free amino acids from the vegetable tannin is also evident from the fact that when the same vegetable tannin materials were added to the medium in which *A. fumigatus* was grown, no increase in the formation of free amino acids in the fungal hyphae could be noticed as compared with the control to which tannins were not added.

The media of the control experiments contained only NO nitrogen where as in those cases in which vegetable tannins were added, the media contained some additional organic nitrogen from the tanning materials. The presence of more amino acids in the fungal hyphae of *A. niger* and *A. flavus* observed in the present study suggests that these fungi were able to utilise the extra organic nitrogen and phosphorous present in the added vegetable tannins for their enhanced growth. The enhanced growth of *A. fumigatus* showed that it was able to utilise the organic nitrogen and phosphorous present in the tanning materials. But, neither the increase in the amounts of free amino acids nor the occurrence of additional free amino acids in the hyphae took place in the case of *A. fumigatus* when it was grown in the test media. The enzyme make up of this species may be such that the accumulation of free amino acids in the fungal hyphae to a larger extent, when grown in test media, did not take place. It is likely that *A. niger* is able to synthesise two additional amino acids leucine and isoleucine from the breakdown products of tannins in the metabolic pathway.

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Fungal Flora in Foot-Wear

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A survey of fungi on footwear was made. *Aspergillus niger* and *A. fumigatus* were found to be predominant species. *A. sydowi*, *A. japonicus*, *A. flavus*, *A. nidulans* and *Penicillium sp.* were frequently seen. Both water and sweat solubles of leather formed good substrates for mould growth, the growth on sweat solubles being more than that on water solubles.

It is well known that the tan liquors[1-11] as well as leathers [12-15] support mould growth. Hence the footwear made of such leathers are susceptible to mould growth. The fungal flora on footwear largely depends on the environmental factors. It may be interesting to know the type of fungi present in ladies footwear since in India ladies usually wear open type of chappals. It is also of interest to know the constituent of footwear that supports the growth of fungi. In the present investigation various moulds present in footwear from Madras city, were isolated and identified. The effect of water solubles and sweat solubles of leather on their growth was also studied.

Material and Methods

The fungi from the footwear were isolated by swabbing the inside of the footwear thoroughly with a sterile moist cotton which was shaken in 10 ml of sterile water in a test tube. Dilutions (10, 100, 1000 and 10,000 times, the original solution) were made and 0.2 ml of each of these dilutions were transferred to sterile petridishes. 25 ml of Czapek's agar medium were added to each and the dishes were incubated for 5 days at $30 \pm 2°C$. The fungi that developed were isolated and identified.

Composition of artificial sweat (g/litre) Urea 1.67 ; Sodium lactate (60%) 100.0 ; Disodium phosphate 0.417 ; sodium chloride 9.75 ; *p*H 6.5.

10 g leather freed from oils and fats by extraction with petroleum ether, was extracted with 200 ml of distilled water or artificial sweat, to get water solubles or sweat solubles, in a shaker (55-60 rpm) for 2 hr. These solubles were filtered and 20 ml portions of these were sterilised in 100 ml Erlenmeyer flasks. Spore suspension of the respective fungi in sterile distilled water, were obtained from 10 day old cultures on Czapek slants and 0.2 ml portions of a uniform suspension served as the inoculum. After incubation for 10 days at 30±2°C the mycelial pads were harvested, washed free of soluble matter, dried at 60°C overnight and weighed.

Results and Discussion

Table 1 shows that the footwear in use contained species of fungi, that were also recorded either on tan liquors or on leathers. These might have

TABLE 1

Earlier and present record of species of *Aspergillus*, *Penicillium* and *Alternaria* occurring on the tan liquor, leather and footwear.

	Tan liquor	*Leather*	*Footwear*
A. sydowi	—	Krishnamurthi[24]	+
A. nidulans	Krishnamurthi[28]	Martin[29]	+
A. japonicus	Krishnamurthi[28]	—	+
A. niger	Krishnamurthi[28] Knudson[1]	Musgrave[14,15] Krishnamurthi[24] Cordon[13,22] Wilson[16]	Greene[30] +
A. fumigatus	George[4]	Orthmann and Higby[9] Cordon[13]	Greene[30] +
A. flavus	Krishnamurthi[28] George[4]	Krishnamurthi[24] Musgrave[15], Cordon[13]	+
A. terreus	Krishnamurthi[28]	Krishnamurthi[25] Musgrave[14] Cordon[13]	+
Paecilomyces varioti	—	Cordon[13]	+
Penicillium sp.	Chambard[3]	Cordon[13,22] Wilson[16], Mitton[1,7] Sen[31], George[4] Raper[32]	Greene[30] +
Alternaria sp	—	—	Greene[30] +

+ reported first time on footwear in India

come from both these sources to the foot-wear, while the possibility of contamination from surroundings cannot be ruled out. The table also shows the presence of *Alternaria sp.* and *Paecilomyces varioti* in addition to those that were found in tanliquors or leathers. The fungi present in footwear varied from person to person. The variation in the fungal flora seen in different footwear revealed that the presence of fungi in footwear depended mainly upon the materials used in the processing of leather and the surroundings in which the footwears were worn. It may also be observed that the presence of fungi did not depend upon the type of footwear, whether shoe, sandal or chappal. From Table 2, it may be seen that *A. niger* and *A. fumigatus* were

TABLE 2

Occurrence of various fungi in men's and women's footwear

Species	Men's shoes	Men's sandals	Women's chappals
A. niger	82	100	100
A. fumigatus	76	100	67
A. terreus	18	—	—
A. sydowi	35	—	—
A. japonicus	24	—	33
A. flavus	6	—	33
A. nidulans	12	40	33
Paecilomyces varioti	6	—	—
Penicillium sp.	35	20	—
Alternaria sp.	6	20	—

Figures are rations of the number of cases where the fungus was present to the total number observed expressed as percentage

predominant. In men's shoes *Penicillium sp. A. sydowi* and *A. japonicus* were frequently found. *Penicillium* sp. , *A. nidulans* and *Alternaria* sp. were also quite frequent in men's sandals. The occurrence of the above species in tropical and subtropical climates on soils and decomposing organic materials is well known[26].

TABLE 3

Growth of fungi on water solubles and sweat soluble of leather (mat wt. in mg)

Source	*A. niger*		*A. fumigatus*		*A. sydowi*		*Alternaria* sp.		*Penicillium* sp.	
	W	S	W	S	W	S	W	S	W	S
Sole leather	16.0	29.2	15.8	39.2	20.4	79.0	16.0	24.8	16.7	19.4
E. I. goat	16.7	29.8	12.3	17.8	16.9	67.3	13.2	35.0	14.8	17.0
E. I. sheep	18.4	31.3	14.2	26.4	18.2	81.2	15.1	26.0	13.4	16.1
Chrome retanned with cutch	10.8	14.4	10.7	16.0	11.5	49.1	11.2	21.1	10.1	16.4
Chrome retanned wattle	10.2	14.7	10.1	15.1	10.2	54.2	11.6	22.4	8.7	13.4

W—Water solubles S—Sweat solubles.

The growth of fungi on water solubles and sweat solubles of leathers is presented in Table 3. It is observed that water solubles from vegetable tanned leathers supported the growth of fungi better than water solubles from res tanned leathers. This may be attributed to low percentage of water solubles, in retan leathers (Table 4). It can, therefore, be suggested that water soluble-

TABLE 4

Water solubles of leather

Type of leather	% on leather
Sole (veg)	11.00
E.I. goat	6.37
E.I. sheep	10.08
Chrome retanned with cutch	3.90
Chrome retanned with wattle	1.61

in leather is one of the main sources of nutrition for fungal growth. The removal of water solubles by soaking, greatly reduced the mould growth[27]. It is also seen that the growth of fungi on sweat solubles was more than that on water solubles. This may be due to the presence of nitrogeneous materials and mineral ions in addition to the usual nontans from leather. It is likely that the worn shoes are more susceptible to mould growth since constituents of sweat can accumulate in them.

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Fungistatic Properties of Stilbenes from Cassia fistula Heartwood

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The presence of 3, 5, 3', 4', 5'-pentahydroxystilbene and a C-glycoside of 3, 5, 2', 4'-tetrahydroxystilbene has been demonstrated for the first time in the heartwood of Konnam (*Cassia fistula*). The fungistatic properties of these two compounds were investigated and reported.

Cassia fistula (Konnam—Common name) is a moderate sized deciduous tree and its bark is used satisfactorily in the tanning of the kips.[1] The chemistry of the different polyphenolics present in the leaves, bark and sapwood of the plant had been studied earlier by Reddy[2] ; it was reported by him that the heartwood contained 10% of tannins but the polyphenolics present in the same remained uninvestigated. It is well known that condensed tannins are located in sufficient quantity primarily in the bark and heartwood of dicotyledonous plants. More often, the compounds present in the heartwood will be different from those present in other parts of the same plant and the compounds will sometimes be quite interesting especially from the standpoint of antifungal and biogenetic considerations.

It is generally known that the woods of some trees decay while those of certain others resist rotting. It was suggested by Hawley *et al*[3], that this unusual resistance of certain woods to decay was attributable to the presence of substances toxic to the fungi. The isolation of 3,5-dihydroxystilbene,[4]

3, 5, 2', 4'-tetrahydroxystilbene[5] and isopropyltropolones[6] which were shown to be toxic to fungi lent support to the above observation. Some parts of Konnam tree trunk obtained for our studies were found to be covered with mildew growth while certain others were free from mildew. Hence a detailed investigation was taken up.

Materials and Methods

Blocks of heartwood, sapwood and bark were sprayed with a suspension of spores of *Aspergillus niger*, *A. flavus*, *A. ochraceous*, *A. terreus* and *Penicillium* sp. The blocks were stored in humid chambers at a relative humidity of 95-100% at 30°C. The development of mildew on these blocks was recorded in Table 1.

TABLE 1

Susceptibility of bark, sapwood and heartwood to mould growth.

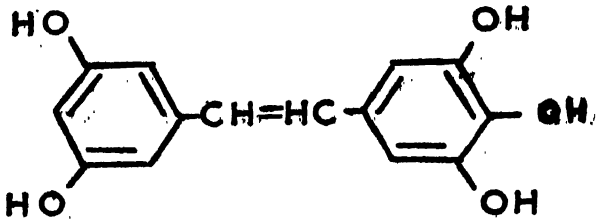
Material	Development of moulds	Remarks
Bark	Heavy growth in one week's exposure	Highly susceptible
Sapwood	Slight growth initially but not progressive	Susceptible
Heartwood	No growth of moulds when exposed for prolonged periods.	Resistant

It was, therefore, evident that the heartwood contained some naturally occurring fungistatic substances which were responsible for this property of the heartwood. A study on the isolation of polyphenolics *vis a vis* the fungistatic substances of the heartwood was therefore taken up.

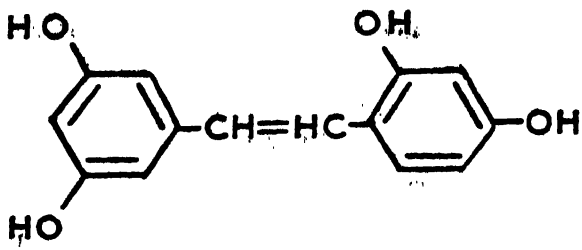
The heartwood collected from a mature plant in April from the Guindy forest, Madras, was sucessively extracted with petroleum ether, ether and ethyl acetate. The petroleum ether extract was found to contain only waxes and was discarded. The ether and the ethyl acetate extracts which were found to contain phenolics on a qualitative examination, were analysed by two-dimensional paper chromatography using solvent systems, (i) composed of 6% acetic acid in the first way followed by butan-2-ol-acetic acid-water (14 : 1 : 5, v/v)[7] in the second way as well as (ii) composed of *n*-butanol-acetic acid-water (4 : 1 : 2.2, v/v) in the first way followed by 2% acetic acid[8] in the second way on Whatman No. 1 chromatographic paper. The chromatograms were developed with (a) ferric chloride and potassium ferricyanide reagent and (b) *bis*—diazotised benzidine[10].

Results and Discussion

The paper chromatogram of the ether extract was found to contain three well-defined spots of which two were identified as (+)-catechin and 3, 5, 3', 4', 5'-pentahydroxystilbene (I). The ethyl acetate extract on a similar exami-



I



II

nation was found to contain only one well-defined phenolic spot which was tentatively identified as a C-glycoside of 3, 5, 2', 4'-tetrahydroxystilbene, the formula of the latter being II.

Ether soluble fraction of the heartwood : Using cellulose column chromatography, (+)-catechin and 3, 5, 3', 4', 5',-pentahydroxystilbene were separated. (+)-Catechin (in traces) was characterised by virtue of its R_f values, infrared spectrum and co-chromatography. The pentahydroxystilbene which has been shown to be the antifungal agent was obtained as a pure compound on crystallisation from 2N acetic acid. The compound showed an intense-blue fluorescence in ultraviolet light and the R_f values and the colour reactions were found to be those of hydrosystilbenes. It gave an intense tranientblue-black colour with ferric chloride. The melting points and the analytical values of the three crystalline acetyl, benzoyl and methyl derivatives of the compound agreed with the values of those of 3, 5, 3', 4', 5'-pentahydroxystilbene earlier isolated by King *et al*[11] from *Vouacapoua macropetala*.

Ethyl acetate soluble fraction of the heartwood

This fraction on purification by ethyl acetate-petroleum ether mixture and subsequent crystallisation from 2% acetic acid afforded a crystalline C-glycoside of 3, 5, 2', 4'-tetrahydroxystilbene which structure was assigned to the same after a preliminary examination. The attachment of the sugar molecule to the stilbene was thought to be through a C-C linkage either at 4- or 2-position of the stilbene. The details regarding the structure of this compound will be published elsewhere. However, the antifungal properties of this compound are reported here.

Fungistatic properties of the 3, 5, 3', 4', 5'-*pentahydroxystilbene* and *the C-glycoside of* 3, 3, 2', 4'-*tetrahydroxystilbene*

Screening of 3, 5, 3', 4', 5'-pentahydroxystilbene and the C-glycoside of 3, 5, 2', 4'-tetrahydroxystilbene was carried out in Czapek medium. These compounds were incorporated in Czapek agar at 1% level and inoculated with *A-niger*. The average results of duplicate observations are presented in Table 2.

TABLE 2

Fungistatic properties of stilbenes

Compound	Observation
3, 5, 3', 4', 5'-Pentahydroxystilbene (1%)	nil
3, 5, 2', 4'-tetrahydroxystilbene C-glycoside (1%)	(+ + +)
Control—Czapek agar	(+ + +)

\+ Poor + + Moderate + + + Heavy

Of these two compounds only pentahydroxystilbene revealed fungistatic properties at 1% level. Since the first screening revealed that pentahydroxystilbene had fungistatic properties, it was taken up for further screening in different media.

Tests were repeated with tan-liquor and Czepek agar media. Varying concentrations of this compound in 2.5% wattle extract tan-luiqor and Czapek agar were obtained. A small amount of acetone was added wherever necessary to facilitate solution of this compound as it was only sparingly soluble in cold water. Results of duplicate trials are given in Table 3.

TABLE 3

Effect of pentahydroxystilbene on the growth of *A. niger*.

Concentration in the medium %	Growth in test media		
	(A) Wattle liquor medium	(B) Wattle liquor medium	(C) Czapek medium
1.00	Nil	Nil	Nil
0.75	Nil	++	++
0.50	+	++	+++
0.25	+++	+++	+++
0.10	+++	+++	+++
Control	+++	+++	+++

(A) Contained 0.1% acetone to help solution of the compound.

(B) Solution of compound achieved by warming up.

(C) As in B with Czapek medium.

Media (A) and (B) contained 0.5% glucose to enhance mould growth.

From the above, it may be concluded that 3, 5, 3', 4', 5'-pentahydroystilbene had fungistatic properties at a concentration of 1% while the C-glycoside of 3, 5, 2', 4'-tetrahydroxystilbene did not inhibit mildew at this concentration. Introduction of a sugar molecule in the compound appears to reduce its fungitoxicity. The resistance of the heartwood to mould attack may be attributed to the presence of the pentahydroxystilbene.

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Effect of Carbon and Nitrogen Sources and Mineral Salts on the Growth and Chromogenesis of Sarcina litoralis, a Halophilic Organism

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Growth of *S. litoralis*, a chromogenic halophilic organism, was significantly increased by glycerine and to some extent by certain other carbon sources. But pigment production was found to be practically unaffected by different carbon sources. Different amino acids influenced the growth of *S. litoralis* to different degrees but heavy growth was obtained only when proteose peptone or vitamin free casamino acid was used. A few amino acids and some other nitrogen containing compounds were found to slightly encourage pigment production while some others retarded carotenogenesis. Proteose peptone promoted both the growth and pigment production. Magnesium salt enhanced growth as well as pigmentation to a considerable extent. Presence of other salts in minute quantities did not appreciably affect the growth or pigmentation.

Requirement of carbon and nitrogen sources for the growth and metabolism varies greatly in different micro-organisms and this variation also affects their chromogenesis. According to Ingraham *et al*[1] glycerol served as the best carbon source for carotenoid production by bacteria. *Mycobacterium phlei* was found to utilise isopropanol and ethylene glycol almost as well as it did utilise glycerol[2] for pigment production. Haas and Bushnell[3] pointed out that a change in the composition of the medium might even change the type of carotene produced. Corpe[4] in a study with several *Chromobacterium* species observed that beef extract, yeast extract, Tryptone (Difco) and a number of other peptones and protein hydrolysates discouraged the pigment formation.

Ingraham and Steenbock[2] further noted that pigment production by *Mycobacterium phiei* was related to the mineral constituents of the medium and was dependent on the concentration of potassium, iron and copper salts. According to Kharasch *et al*[5] variable concentrations of biologically important metalslike manganese, copper and iron might influence both the growth of and pigment production by micro-organisms. The present work deals with the effect of different carbon and nitrogen sources and certain mineral salts on the growth and carotenogenesis of *S. litoralis*, a halophilic organism commonly found associated with 'red heat' on salted hides and skins.

Materials and Methods

Sarcina litoralis 90-R5 was obtained from G. B. Landerkin, Microbiology Research Institute, Canada Department Agriculture, Ottawa, Canada.

To find out the growth and carotenogenesis of *S. litoralis* a number of compounds representing different sources of carbon and also of nitrogen were added to the control medium and the growth and pigment production obtained in each case were compared with those obtained with the control medium. The basal medium contained the following (g/l) : $(NH4)_2\ SO_4\ 7H_2O$-10g, asparagine—0.8g, $Mg\ SO_4\ 7H_2O$—20g, $FeCl_3\ 6H_2O$—0.02g, KH_2PO_4—12g, $K_2\ PO_4$—1.2g, NaCl—200 g, *p*H was adjusted to 6.7—6.8 with a Beckman *p*H meter and the following carbon compounds were added separately to the above basal medium taken in Erlenmeyer flasks. Glucose, lactose, sucrose, fructose, xylose, arabinose, mannitol, galactose, maltose, starch (soluble), glycerine and sodium gluconate (all in the proportion of 1%) sodium acetate and sodium succinate (0.68g/100 ml) and sodium citrate (0.5 g/100 ml). The *p*H of the medium in each flask was again adjusted to 6.7—6.8 and 1.2% agar (B.D.H. powder) was then added. The media were steamed in a sterilizer for 30 min. and then poured on (20 ml) to sterile petridishes (10 cm).

The effect of various nitrogen sources was studied using the same basal medium with 10g of glycerine in the place of asparagine. The following amino acids and other nitrogen compounds were added to the basal medium in the following proportions (g/100 ml) : DL—aspartic acid (0.355), L—glutamic acid (0.393), L—leucine (0.350), DL—isoleucine (0.350). DL—alanine (0.238), L—cystine (0.321). L—cysteine HCl (0.472), glycine (0.201), L—histidine HCl (0.171), DL—methionine (0.398), DL—asparagine (0.200), urea (0.08), sodium nitrate (0.227), Proteose peptone (Difco)(0.100), vitamin free Casamino acid (Difco) (0.100). As before *p*H of the medium was adjusted to 6.7—6.8 ; agar (1.2%) was then added and the media were steamed and poured on to the plates.

To study the effect of amino acids, 20 amino acids were divided into four groups of five amino acids and were incorporated in the media. Growth of *S. litoralis* on media containing amino acid mixtures was examined in a preliminary study and 10 amino acids belonging to two groups were screened out. The remaining 10 amino acids and other nitrogen containing compounds are used in the present study.

To study the influence of mineral salts, the basal medium used for the assessment of nitrogen sources was modified by the addition of DL—methionine (3.98g) and by excluding magnesium and ferric salts The following salts were added to the basal medium (g/100 ml) : $MgSO_4\,7H_2O$—0.5, 1.0 and 2.0, $FeSO_4\,7H_2O$—0.001, $CaCl_2\,2H_2O$—0.01, $MnSO_4\,4H_2O$—0.00001, $CuSO_4$, $5H_2O$ — 0.0001. The *p*H was adjusted and finally poured on to the petridishes as before.

Cells of *S. litoralis* grown on a basal medium at 37°C for 2 weeks were taken up in 20% sodium chloride solution and centrifuged. Cells were then resuspended in 20% sodium chloride solution and 0.2 ml of this suspension was used as the inoculum. Growth and pigment production by *S. litoralis* were estimated according to the method reported earlier[6]. A filter of 660 mμ was used in a Klett-Summerson photoelectric colorimeter to estimate growth turbidimetrically and expressed in terms of optical density and a filter of 520 mμ for measuring the pigment and turbidity. The difference of these two readings gave the measure of the pigment and was expressed in terms of optical density per unit mass of cells. It was further reported that a slight variation in value (*e.g.*, 0.0022) will indicate a variation of about 5% of the total extractable red pigment. In the present study variations in the optical density of about 0.02 in the case of growth and about 0.001 in the case of pigment were considered insignificant. Both the growth and pigment production are expressed in terms of optical density.

Results and Discussion

Synthetic media containing different carbon and nitrogen sources and metal salts were inoculated with the cells of *S. litoralis* and the culture plates were incubated at 37°C for 3 weeks. Cells grown on culture plate were scraped carefully and taken up in 10 ml of distilled water. Growth and pigmentation were then estimated and the average of four values are presented in Tables 1 2 and 3.

TABLE 1

Effect of carbon source on the growth and chromogenesis of *S. litoralis*

Carbon source	Growth (O.D.)	Pigment per standard turbidity (O.D.)
Glucose	0.134	0.0568
Lactose	0.087	0.0547
Sucrose	0.092	0.0557
Fructose	0.071	0.0553
Xylose	0.064	0.0556
Arabinose	0.063	0.0540
Mannitol	0.031	—
Galactose	0.122	0.0565
Maltose	0.105	0.0559
Starch	0.075	0.0558
Glycerine	0.350	0.0558
Sodium acetate	0.062	0.0551
Sodium tartrate	0.060	0.0554
Sodium succinate	0.070	0.0540
Sodium gluconate	0.060	0.0557
Control	0.064	0.0540

TABLE 2

Effect of nitrogen source on the growth and chromogenesis of *S. litoralis*

Nitrogen source	(Growth (O.D.)	Pigment per standard turbidity (O.D.)
DL-Aspartic acid	0.900	0.0498
L-Glutamic acid	0.124	0.0563
L-Leucine	0.130	0.0685
DL-Isoleucine	0.106	0.0571
DL-Alanine	0.015	—
L-Cystine	0.176	0.0691
L-Cysteine HCl	0.165	0.0684
Glycine	0.076	0.0575
L-Histidine HCl	0.020	—
DL-Methionine	0.530	0.0677
DL-Asparagine	0.204	0.0558
Sodium nitrate	0.104	0.0685
Urea	0.148	0.0696
Proteose peptone	3.960	0.0727
Casamino acid	3.340	0.0691
Control medium	0.160	0.0667

TABLE 3

Effect of different mineral salts on the growth and chromogenesis of *S. litoralis*

Mineral salts	Growth (O.D.)	Pigment per standard turbidity (O.D.)
Magnesium sulphate		
(0.5%)	0.296	0.0624
(1.0%)	0.326	0.0650
(2.0%)	0.336	0.0663
(2.0%)	0.336	0.0663
Ferrous sulphate	0.140	0.0620
Calcium chloride	0.124	0.0633
Copper sulphate	0.134	0.0647
Manganese sulphate	0.192	0.0650
Control	0.116	0.0598

It appears from Table 1 that, in general, different carbon sources did not appreciably affect the growth and pigmentation of *S. loitralis*. It was only glycerine which encouraged the growth to a great extent and glucose, galactose and maltose to some extent. However, the pigment production was more or less the same even in these cases. Table 2 shows that the growth of *S. litoralis* was increased slightly by DL—asparagine, moderately by DL—methionine and DL—aspartic acid and considerably by proteose peptone and vitamin free casamino acid. Pigment production had increased to some extent with L—leucine , L—cystine, L—cysteine HCl., sodium nitrate, urea and casmino acid but appreciably by proteose peptone.

Results obtained in Table 3 indicate that 1-2% magnesium sulphate heptahydrate, enriched both the growth and chromogenesis of *S. litoralis*. All the other mineral salts tested did not, however, encourage pigment production to any appreciable extent.

Growth of *S. litoralis* has been accelerated by glycerine while other carbon sources did not appreciably influence it. Regarding carotenogenesis carbon sources did not play any significant role.

Ingraham and Steenbock's[8] observation that glycerine was the best carbon source for the production of carotenoid pigments in bacteria could not, however, be supported in the case of *S. litoralis*. Schopfer and Grob[7] found sodium acetate to synthesise carotene in *Phycomyces*, but in the present study sodium acetate was found to have no influence on the carotenogenesis of *S. litoralis*. Baxter [8] also reported that glycerol or acetate which were supposed to be the precursors of carotenoid pigments, did not increase pigment production by *Halobacterium salinarium*, a halophilic organism. It appears that DL—methionine enhances the growth to a considerable extent without affecting the pigment production. On the other hand L—leucine, L—cystine, L—cysteine, sodium nitrate and urea may slightly increase pigment production without affecting the growth appreciably.

DL—aspartic acid and DL—asparagine, favor growth but retard pigmentation. DL—alanine and L—histidine are found to retard the growth of the organism.

Proteose peptone promotes heavy growth of and maximum pigment production by *S. litoralis* followed by vitamin free casamino acid.

It was reported by Goodwin and Lijinsky[9] that carotenogenesis in *Physomyces blakesleeanus* was stimulated by L-leucine and L-valine They further suggested that both the amino acid could furnish a C5 residue $\overset{C}{\underset{C}{>}}C—C—C$ which might serve as an active repeating unit in the synthesis of carotene In the case of *S litoralis*, however, L-leucine or L-lavine (examined separately) do not show any appreciable stimulating effect on carotenogenesis Baxter[8] made a similar observation while studying the carotenogenesis of *H. salinarium*.

A concentration of 2.0% of magnesium sulphate is required for maximum growth and pigmentation of *S. litoralis*. According to Brown and Gibbonse[10] 0.1-0.5 M magnesium ion was necessary for maximum growth of halophilic bacteria. Katzneleson and Lochhead[11] used 2% magnesium sulphate for the growth of halophilic bacteria. Other mineral salts except manganese do not appreciably favour growth under the experimental conditions. According to Sehgal and Gibbons[12] ferrous sulphate enhanced the growth of the halophilic organisms. This variation in observation may, probably, be due to the fact that a liquid medium has been used by Shegal and Gibbons, where as a solid medium has been used in the present study. It may be noted, that the mineral salts tested increase pigment production to some extent. It is recognised that curing salt of marine origin contains Mg, Ca and Fe salts as impurities and as such will help in the development of ' red heat '.

Acknowledgement

Thanks are due to Dr. Y. Nayudamma, Director, Central Leather Research Institute, for his interest in the work and for permission to publish the results.

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DISCUSSION

SESSION I

S. N. Sen (*Central Leather Research Institute, Madras*) : Is the problem of red pigmentation of chrome blue very common or only of rare occurrence ? What precautionary measures would Dr. Orlita suggest ? Do importers insist on Indian exporters following any specification for the exported blue chrome leather ?

C. Halamek (*National Research Institute for Shoe Leather and Allied Industries, Gottwaldov, Czechoslovakia who presented the paper of Dr. Orlita*) : This problem is not very serious. Preservatives in specified concentrations could be added to the fat liquor and this will take care of it.

R. Thanjan (*Development Officer, Leather, New Delhi*) : No specifications were imposed on chrome blue.

C. V. Subramaniam (*University Botany Laboratory, Madras*) : Is there in the tan liquor anything like a succession of microorganisms ? Is it possible that depletion of substrates could take place when microbial growth may come to a stop ?

R. Bhaskaran (*Central Leather Research Institute , Madras*) : Order of microbial infection has been studied on leather. Usually *Aspergilli* appear on leather first followed by *Penicillia*. Certain bacteria have been shown to follow a succession of fungi utilizing the breakdown products in the substrate. This can probably be termed symbiosis in some cases. However a detailed study is necessary in this field of succession of microflora in vegetable tanning liquors.

F. Verzer (*Institute for Experimental Gerontology, Basel, Switzerland*) : Determination of collagenase may help to find bacterial degradation. The conditions of microbial succession in different stages of leather manufacture may also be taken into consideration.

R. Bhaskaran: Denaturation of the materials such as collagen aids in promoting bacterial growth ; pure native collagen may not support growth of most bacteria since the enzyme collagenase has been found only in a few specified species of bacteria. In tanned leather it is the other non-tan substances such as water solubles, fats, etc., that support microorganisms ; tanned collagen is not attacked by fungi.

Y. Nayudamma (*Central Leather Research Institute, Madras*) : Dr. Halamek, what is your opinion on the letting out of tan liquors (effluents) particularly from chrome tanneries in the fields ?

C. Halamek : It is obligatory to purify the waste waters thoroughly before they are let into the rivers. The settled dried sludges of waste water are used as manure. The sludges obtained from vegetable or synthetic tannins did not have any disagreeable effects, but the

presence of trivalent chromium was deleterious. This chromium accumulates in the soil and it is inimical to plant growth. Hence it may be added to soils only intermittently say, once in 2-5 years.

C. Halamek : Mr. Kamath, we have also worked on fungi in footwear, on moisture content and history of footwear. A statistical study may have to be done if the interpretation is to be meaningful.

D. H. Kamath (*Central Leather Research Institute, Madras*) : We investigated the fungal flora from shoes in regular use. Some more data are needed for a statistical study and we have this in mind.

T. S. Sadasivan (*University Botany Laboratory, Madras*) : What metallic ions are present in skin and leather ? Perhaps you are aware of the new subject biogeochemistry? It has been found from leaf analysis of trees in an area in Africa, that lead was present in the leaves and it was suggested that the soil may contain a strata of lead and this proved true. In the Nutritional Research Institute, Hyderabad they have found that many metallic ions are excreted in the sweat of human beings.

S.C. Nandy (*Central Leather Research Institute, Madras*) : Most of the metallic ions are present in raw hides and skins.

Y. Nayudamma : Half of the elements in the Periodic Table are present in leather.

B. K. Patnaik (*Regional College of Education, Bhuvaneswar*) : Mr. George, you stated that tannin increased total amino acid content in a few species of fungi. Do you think that protein synthesis is also influenced ?

E.C. George (*Central Leather Research Institute, Madras*) : It has not been studied. It may be influenced.

G. S. Rama Iyer, (*Central Leather Research Institute, Madras*) : Whether growth of fungi in footwear is influenced by adhesives, glue starch paste and natural adhesives as rubber latex, etc. ? When footwear got soaked these may also seep into leather, and influence growth of moulds on leather.

R. Bhaskaran : All substances *e.g.*, even threads coated with sizing in addition to water solubles and fats support mould growth.

C. Halamek : The fungi in footwaer not only come from the processing of raw materials but also during use. Certain mercury compounds have been used as fungicides but they have not been widely accepted as mercury can be a contact poison for the humans and is generally considered as a health hazard.

S. Bangaruswami (*Central Leather Research Institute, Madras*) : Certain biological preparations are freeze-dried. Can hides and skins be freeze-dried ?

S.C. Nandy : Studies have been made in this regard. Freeze-dried pelts wet back very well, but lose the feeling of pelt to a large extent. Ice crystals are formed and cause defects on grain and the leather gets a peculiar rubbery feel. If stored in moist condition, they again pick up mould growth. Covering all aspects, freeze-drying is not very encouraging.

Y. Nayudamma : A consignment of shoes was had to be burnt at the New York airport due to mouldiness. What do you suggest for prevention of such occurrences ?

R. Bhaskaran : Different leathers and threads used in the manufactured shoe can be treated with fungicides : usually it is more difficult to treat manufactured shoes. Unless this is done mildew develop when these shoes are exposed to a humid atmosphere. Alternatively the moisture in the atmosphere around the shoes may be reduced below 65% RH with the aid of desiccants like silicagel etc., enclosed in thick polythene bags.

T. S. Sadasivan : The use of nickel ions, ultraviolet irradiation, inclusion of silicagel in sealed plastic bags containing shoes so as not to absorb moisture may prevent fungal contaminations. Recent advent of many organometallic compounds such as silver and mercury as mentioned by C. Halamek may be tried. Some of the dithiocarbamates could also be used (Mercaptan, nabam, etc., are powerful antifungal agents). Silver is costly but nickel compounds are both effective and comparatively cheap. For fungal infections of tea bushes they are now widely using nickel chloride as a protectant. The role of nickel as an antifungal agent in leather processing and finishing, therefore merits investigation. Ultraviolet irradiation could be done as a routine final treatment when the shoes are packed in sealed plastic containers. If found effective experimentally, commercial treatment in warehouses by having a battery of UV lamps and the packed shoes or other leather wear moving slowly in conveyer belts can minimise the fungal flora of the packet and the silicagel will keep the humidity down to a level of 10% or lower, when any spore not knocked down by UV treatment will be unable to germinate.

K. P. Chandrasekaran (*Madras Veterinary College*) : Fungi are found everywhere. Toxicogenesis of fungi in footwear may be investigated. For example, *Aspergillus fumigatus* and *A. flavus* produce potent toxins toxic at 1 p.p.m. This toxin has been found to cause death in ducklings. Aflatoxin also causes death of buffalo calves due to contamination of oil cake. Isolation of toxigenic fungi is also important.

Technical Session II

Biological Study of Unborn Goat Skin

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A study on the development of skins of goat foetuses at different prenatal stages showed that follicle initiation occurred as early as two months after gestation ; ' course ' or ' long ' hair follicles developed much earlier than ' fine ' or ' short ' variety. A progressive development of other skin tissues is described in this paper.

Goat, the ruminant quadruped belonging to the genetic group of a *Capra hircus* is found particularly in Europe and Asia. India alone has got a goat population of about 47 millions. There are about 23 breeds of goat but pure breeds are rare ; they get mixed up and produced cross breed animals, commonly known as ' Desis ' in India. The gestation period of Indian goat is about 6 months, delivering off-spring varying between 1 and 3 usually 2 in number at a time.

Histological study has revealed that Indian goat skin possesses some inherent tissue characteristics which are largely influenced by a number of factors like sex, breed, place of origin, etc.[1] The above findings are based on the study of skin samples collected from adult animals ; but giving no information about the gradual development of different tissues during the early stages of animal growth. Most of the investigators,[2-6] confined their studies on sheep skins in the prenatal stages, more particularly on the nature of development of follicles, wool and other epidermal structures. Later, Lyne and Margaret[7] also studied the growth of hairs, glands etc., in 33 cattle foetuses ranging from 68-274 days after gestation. Lack of proper information in respect to goat skin development during prenatal stages geared to undertake the present study from the histological point of view on skins collected from foetuses having age range from 8 weeks to 6 months (at birth) after gestation.

Materials and Methods

20 skin samples of different age group *e.g.*, 3 skins of 2 months (9-11 weeks), 3 skins of 3 months (12-15 weeks), 4 skins of 4 months (16-20 weeks,) 5 skins of 5 months (21-25 weeks) and 5 skins of 6 months (26 weeks-birth) age were taken for the present study.

Flayed skins (free from shanks, tail etc.) were washed, blotted and weighed. For measuring natural grain tightness, rectangular pieces measuring 1.5×1.5 cm were collected from location 3 cm from the tail root and 1 cm from the backbone. Remaining portions were preserved in 7% formol saline. For histological study, small pieces were cut from the preserved skins from location 1 cm from the tail root and 1 cm from the backbone and sections were cut in freezing microtome. Histological techniques[8-9] as applied to the study of hides, skins and leather were followed and microscopical measurement was made as and when found necessary.

The method followed by White and Caughley[10] was used to measure the natural grain tightness and the results are expressed as the percent shrinkage of the area of the grain in relation to that of the corium below, after separating them with freezing microtome. In all the cases average data are presented.

Results and Discussion

Development of the skins : The skin of animal is not homogeneous but is composed of several varieties of tissues and its structure undergoes several folds of changes in weight and in thickness during the early foetal stage till birth. Data obtained are given in Table 1.

TABLE 1

Changes in weight and thickness of skin during early foetal stage

Age Group (Months)	Skin weight (g)	Thickness of the skin (mm)
2	40	0.73
3	67	0.87
4	91	1.12
5	107	1.29
6	147	1.48

The above data clearly indicates that the skin weight increases progressively during the foetal period which is definitely due to the gradual development of different tissues from their early cellular stage resulting in an increase in thickness and size of the skin. Lyne and Margaret[7] in their study with cattle foetuses also noticed a progressive increase in skin thickness throughout the foetal period.

Epidermis, the outermost layer of the skin is quite distinct in the early foetus of 8 weeks old but it undergoes some morphological changes in the later pre-natal stages. The thickness however varies only between 0.028 and 0.035 mm and comparatively a greater thickness is noticed when the hairs begin to emerge out of the skin surface. This seems to be quite reasonable because from the initiation of hair folilcle to hair growth, the epidermis changes rapidly giving rise to the growth of follicles first and hair later. Throughout the entire foetal period, two strata of epidermis are distinct, the upper horny stratum, the 'corneum' and the lower actively growing stratum the 'germinativum': the intermediate stratum *i.e.*, 'granulosum' as reported by early workers[1] in adult goat skin seems to develop much later. A considerable amount of mucoid substance is found associated with the cells of the bottom stratum at this stage. The pigment granules often associated with the bottom stratum are practically absent in the early foetal stage but a progressive accumulation takes place with the development of the foetuses. This is possibly due to the gradual modification of the epithelial cells, some of which initiate the development of dendritic cells from which pigment granules are produced.

Growth of Hair Folicles, Hairs and Glands. It is the epidermis, particularly the cells of the bottom stratum which gives rise to the growth of the follicle first and hair later. Initiation of follicle occurs with the localised thickening of the bottom layer of the epidermis and its gradual dip down in the derma or corium. The development of hair from its early stage *i.e.*, follicle, to its appearance from the outer surface have been described to occur in a number of stages [11-13]. Present study indicates that hair follicle initiation in goat skin occurs much earlier than two months of gestation. However, in two months old foetus most of the follicles are noticed at the earliest stage of hair growth *i.e.*, follicle plug (Fig. 1 and 2) and only a few are noticed in much advanced stage of follicle development. At this stage no hair is visible but is just noticeable after 3 months inside the follice, and nothing is visible on the skin surface (Fig. 3). Later, with further development the hair is pushed out of the skin. Fat and sweat glands which are closely associated with the hair also start growing simulatneously. In the former a progressive accumulation

of fat cells takes place in the gland sac during the foetal period increasing its size, and its location in the skin varies only between 0.10 and 0.13 mm below the surface. Sweat gland, on the other hand, appears as a small hanging loop connected to the mouth of the follicle by a narrow duct (Fig. 2) but shows very little change in size during the entire foetal period. It, however, gets bigger and coily after birth. This clearly indicates that during the foetal stage sweat gland remains inactive and starts functioning after birth of the animal and its subsequent participation in the thermostatic mechanism of the body temperature causes its proper development. The depth of this gland during the entire foetal period varies between 0.27 and 0.53 mm below the surface.

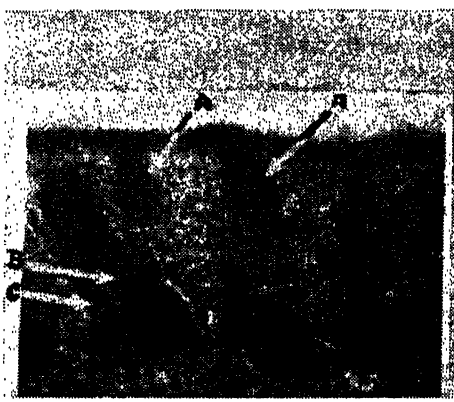

Fig. 1. 8 *weeks old foetus*

A—Follicle plug

B—Much developed hair follicle

C—Sweat gland (×105)

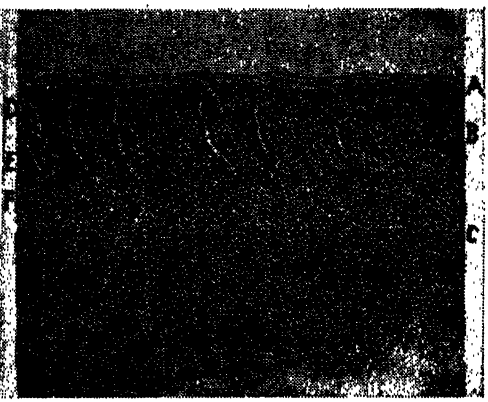

Fig. 2. 11 *weeks old foetus*

A—Epidermis; B—Grain

C—Corium; D—Fat gland

E—Sweat gland; F—Hair bulb (×41)

It has also been reported earlier[1] that goat skin possesses two types of hair, namely fine or short hairs and coarse or long hairs. The present study has revealed that these do not develop simultaneously in the prenatal stage ; 'long' or 'corase' hair follicles and hairs develop much earlier than the former. The 'fine' or 'short' hair follicles perhaps remain in static condition without any development of hair during the early foetal period, but the development of fine hair probably occurs in much advanced foetal stage or

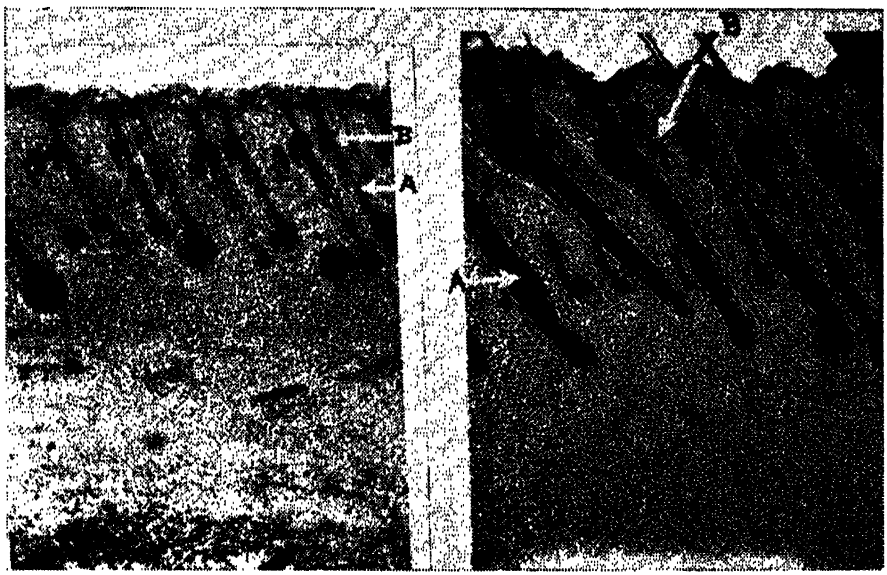

Fig. 3. 3 *months old foetus*

A—Newly developed hair

B—Fat gland (× 41)

Fig. 4. 6 *months old foetus*

A—Coarse hair

B—Fine hair follicles (× 41)

after birth (Fig. 4). The ' long ' or ' corase ' hair follicles, however, follow the same sequence in their arrangement as in the skin of adult animal even in the early foetal stage *i.e.*, they are arranged in groups of three but in this early foetal stage ' fine ' or ' short ' hair follicles are practically absent (Fig. 5). Their presence is noticed only after the foetuses has attained an age of about 5 months. At this stage the grain surface shows both ' coarse ' as well as ' fine ' hair follicle pores with the same sequence of arrangement as in the adult goat skin. (Fig. 6).

The ' coarse ' or ' long ' hair follicles start developing as early as 2 months after gestation and its number (density) seems to get fixed during the early foetal

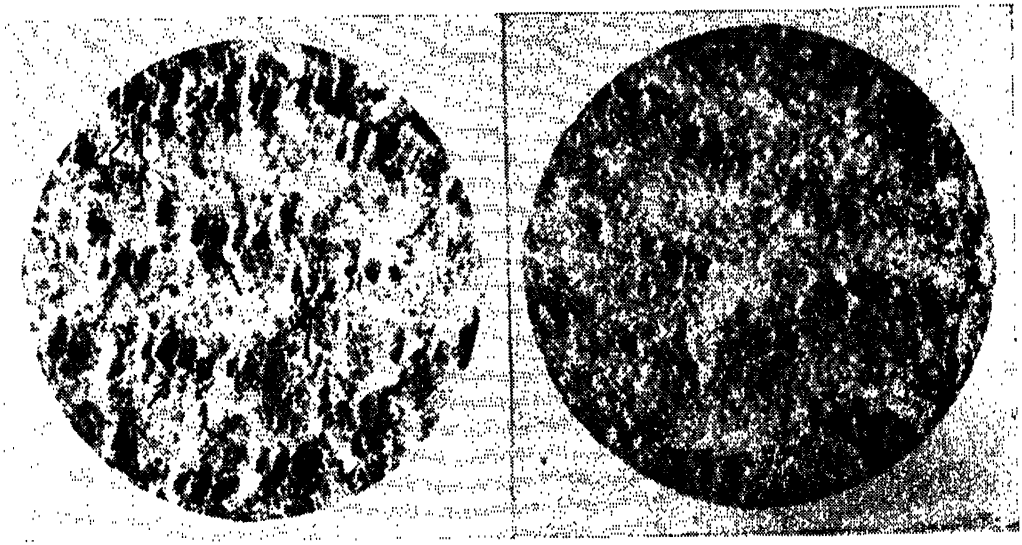

Fig. 5. *Grain surface patterns of* 3 *months old foetus showing coarse or long hair follicle pores in trio-groups* (× 60)

Fig. 6. *Grain surface pattern of* 6 *months old foetus showing 'fine' or 'short' follicle pores along with 'coarse' or 'long' hair follicle pores* (× 41)

stage but with the gradual development of the foetus, the skin area gradually increases with the consequent decrease in coarse hair density as is evident from Table 2.

TABLE 2

Hair growth during foetal stage

Age group (Months)	No. of coarse or long hair per sq. cm.	Depth of the hair follicle or hair (mm)	Diameter of hair (Coarse) (mm)	Inclination of hair or follicle with the surface
2	6270*	0.32	—	64°
3	4615+	0.59	0.012	63°
4	3500	0.79	0.026	40°
5	2869	0.87	0.029	46°
6	2343	0.86	0.041	47°

*Follicle only +Follicle and hair

It is also evident from the above data that the depth of the follicles and hairs increases during the early foetal period till 5 months, after which they remain more or less stationery. The diameter of the hair also increases during the foetal period. A comparatively higher inclination is noticed at the early developmental stages of the hair follicle. This change may be explained by the fact that at the early stage of follicle development, the follicle plug dips down in the derma or corium more or less vertically but in the later stages this inclination comes down probably by the combined effects of the exposed hair as well as by the tension caused due to the increase in surface area with the progressive development of the foetuses.

Development of the Grain and Corium proper. The corium is divided into two layers, namely grain and corium proper; in the former the collagen fibres are extremely thin, short and they are compactly woven holding the structures like hair, hair follicles, glands etc., together with some elastic fibres whereas the corium proper is predominantly composed of collagen fibres of variable sizes. The development of grain is quite pronounced in the prenatal stage as is evident from Table 3.

TABLE 3

Development of grain during foetal stage

Age Group (Months)	Total thickness* (mm)	Grain thickness (mm)	Grain of total thickness (%)	Corium proper of total thickness (%)
2	0.73	0.28	38.3	57.9
3	0.87	0.43	49.4	44.6
4	1.12	0.72	64.2	33.3
5	1.29	0.74	59.3	40.5
6	1.48	0.74	48.6	49.5

*Total thickness includes epidermis, grain and corium proper.

From the above data it is noticed that the development of grain occurs rapidly during the early foetal stage till 4 months during which period the corium proper remains more or less the same. But after this period, the grain layer becomes more or less stationary whereas the corium proper develops at a faster rate till birth. It still remains to be a matter of investigation whether further development of the grain thickness occurs after birth. The collagen fibres of the skins of 2 months foetuses are extremely thin and short; in many

cases it is really difficult to differentiate them under microscope of moderate high magnification but gradually become longer, thicker and closer in texture with age during the pre-natal stage.

The natural grain tightness which is actually the measures of tension by which the grain layer is held over the corium proper increases progressively during the entire foetal period as indicated in Table 4.

TABLE 4

Natural grain tightness during foetal stage

Age group of the foetus (Months)	Grain tightness (%)
2	—
3	4.6
4	6.0
5	9.6
6	10.0

The natural grain tightness of Indian goat skins (adult at the butt usually varies between 12 and 25% ,and its low value in the present study may be due to the improper development of the collagen fibres in the prenatal stage. Many of them are in cellualr state and they do not naturally possess the same physical properties as those of developed ones. Moreover, by nature, the grain at this stage is held rather in a loosely stretched condition to give room for further development of the skin area with age. It is also noted that the grain elastic fibres which are responsible for the elasticity of the grain cannot be recognised in the early foetal stage. They could, however, be differentiated at the age of 5 months in the prenatal stage and this may also partly explain the cause of lower natural grain tightness in the early foetuses. From the above findings, it may also be concluded that the elastic fibres of the grain in goat development much later than the collagen fibres but it is not possible to say from this study whether they are formed by the conversion of collagen fibres as has been mentioned by many investigators [14, 15, 16, 17].

The present investigation is based, only on the histological point of view and the effect of breed or sex has not been taken into consideration. Moreover, the chemical changes occurring in skin composition during its early development have not also been taken into account.

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Use of Infrared Rays for Drying Skins for Conservation

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Drying of sheep, yearling and lamb skins by infrared rays was investigated and was found to be much faster than by artificial drying with warm air or by ordinary drying in natural environment. Infrared drying was done in a drying chamber equipped with bright infrared ray lamps of 125 watts and with ventilators for blowing out of the separated moisture from the skins. Temperature control was effected by thermocouple placed on the surface and in the skins. The drying of raw skins, however, could not be completed by this method as fast as with other products of industry. This was due to the difficulty in warming up the water, present deep in the skin, to a higher temperature without causing the possible destruction of the collagen in the derma. Depending on the availability of electricity at a cheaper rate this method of drying skins could be made acceptable since it provides possibilities for fast drying and early transport of skins to the tanneries.

Drying is a widely applied method for conservation of skins. Big rooms are needed for drying under natural conditions. Bad weather influences the drying process, and lowers the quality of the skins. On the other hand, lamb and sheep skins usually come in bulk during early spring and autumn, when the atmospheric conditions are least favourable for drying. Apart from this, in many meat packeries there are no adequate stores for drying and the skins are either atmospherically dried outdoors or are being salted. Salting, however, has many negative sides and is not favoured for these raw materials.

Different methods exist for drying skins under artificially created conditions. One of these is drying under a flow of warm air and artificial ventilation which shortens the drying period 12 to 15 times in comparison to natural conditions of drying[2].

Another possibility for artificial drying of skins and accelerating the drying process is irradiation of the skins with infrared rays. These rays are known for their high penetrating effect and accelerated drying results. Infrared rays have already found their place in some phases of skin processing[1]. There are no communications, however, for the use of infrared ray for drying of raw animal skins.

Having in mind the advantages of infrared rays we have endeavoured to verify whether they could be successfully used for drying raw skins in artificial drying houses.

Materials and Methods

To verify some basic problems, experiments were made for drying separate pieces and whole skins. In these experiments the following aspects were taken into consideration : power of the lamps, air temperature, air temperature contacting the skin, temperature of the irradiated skin in depth, distance of the irradiated skin surface from the lamp, distribution of the rays upon the skin surface, angle under which the rays contact the skin, relative humidity of the air around the skins, method for preparing and submitting the skins to the rays, period of the drying and quality of the dried skins under different conditions (raw conserved skin, tannery processing and quality of the ready processed skins). In these experiments 16 lamb skins and 12 sheep skins as well as skin pieces were used. The lamb skins were with the wool and the sheep skins with wool cut up to 5 cm or with full wool.

The majority of the skins were submitted to the action of the infrared rays with a drying temperature in depth of the skin of 37-30°C and temperature of the contacting air 27-29°C that is, temperatures which could not deteriorate the collagen of the skins within the period of irradiation[2]. A small number of skins were submitted to higher tempreature which gave upto 52°C in depth, and 38-40°C of the contacting air respectively. These temperatures were obtained by direct irradiation of the skin surface by light irradiation units (lamps) of 125 W[1], placed at a predetermined distance from the skin surface and at a perpendicular direction to the central beam. Heaters of 250 W or 62.5 W were inadequate, firstly because of the possibility of preheating the skins and eventual damage and secondly because of their weaker drying effect.

Recording of the temperature in depth of the skins was made by a system of thermocouples and of the contacting air by a thermometer contacting the skin surface. In the drying experiment it was observed that upto one hour the

temperature of the contacting air was higher by 4-6°C than the temperature in depth of the skin itself, and in the course of drying the later was gradually augmented with a possible difference of 12-14°C. This is illustrated in Figs. 1 and 2.

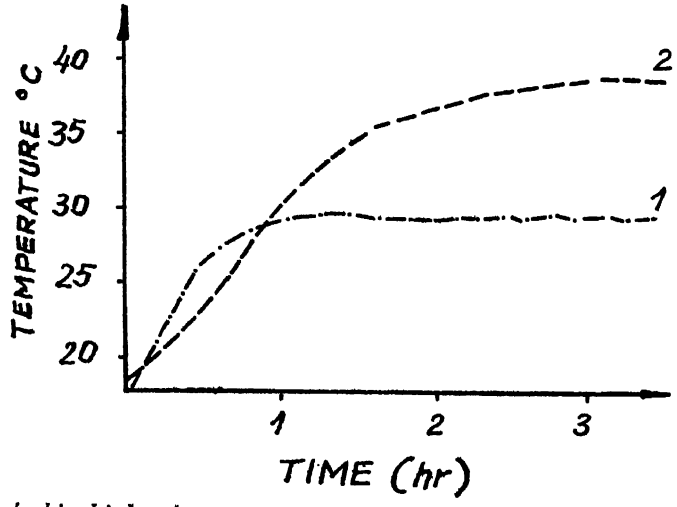

Fig. 1. *Lamb skin dried at low temperatures* : (1) *Temperature* (°C) *of the air in contact.* (2) *Temperature* (°C) *in depth of the skin.*

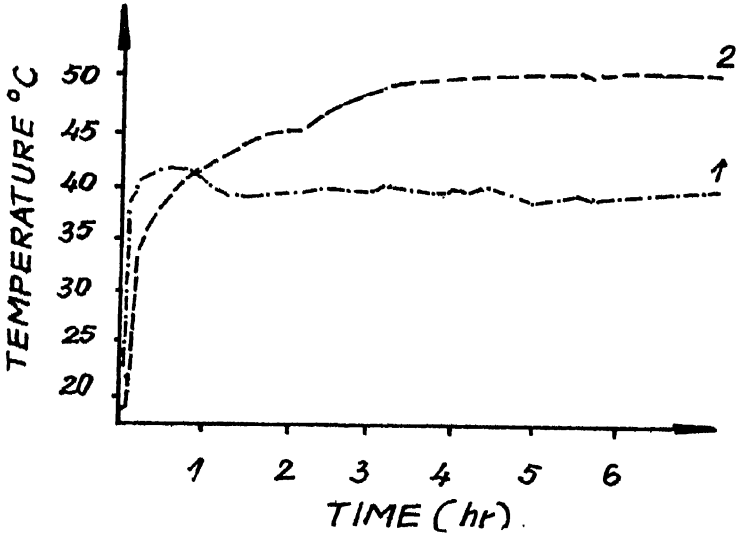

Fig. 2. *Lamb skin dried at higher temparatures* : (1) *Temperature* (°C) *of the air in contact.* (2) *Temperature* (°C) *in depth of the skin.*

The necessary relative humidity for drying the skins was adjusted by ventilators and was recorded by a hygrograph. During the experiments it varied between 50 and 35%.

During drying some of the skins were hung freely, others were put on lath, spread on wooden form or laid on grid frames. In all the cases the irradiation was effected on the innerside of the skins.

The majority of the skins were dried with a small movement of the power unit and others with no movement at all. The drying process continued until the skin attained the qualities of a dried product. This was verified by comparing the initial and the final weight of the skin, and by estimating the moisture content retained by the skins.

The skins had an appearance of well dried product with a light gloss on the backside and with well preserved wool. The water content measured at standard sites was little higher than that of dried skins. Prolongation of the drying process to attain the standard water content was dangerous because over heating might occur, thus damaging the collagen. Samples of skins without wool placed in clean water (without those dried at temperatures of 50-52°C in the depth of the skin) showed a normal fresh skin outlook with an increase in weight of 315% to dry substance[2].

In the factory processing the experimental skins those dried at lower temperatures *i.e.*, 37-39°C in depth of the skin showed a normal outlook, while skins dried at higher temperatures did not attain it. Differences in the processing of dried skins were shown only with these skins.

During factory processing it was observed that skins dried at lower temperatures showed better quality. In certain places the processed skins, dried at high temperature, appeared to be thin and had uneven surface leading up to holes which were due to deterioration of the collagen.

Physicomechanically the skins dried at low temperatures showed a stretching index of 0.83-1.24 for the lamb skins and 1.07-1.42 for the sheep skins; elongation at 0.5 kg/sq mm was 28-58%. Skins dried at higher temperatures showed stretching indexes for the lamb skins 0.38-0.79 and for sheep skins 0.56-0.94.

The good results obtained with the preliminary experiments gave us ground for further experiments in a laboratory drying chamber with increased number of lamb and sheep skins.

The drying chamber was made of wood with the following dimensions : length 2.5 m, width 1.25 m and height 2 m. The inside walls were covered with thin tin foil. The lambs were mounted on the ceiling in chequered design with beams directed towards the floors. During drying, the skins were placed upon nets, mounted on movable frame and were kept 1.10 to 1.30 m away from the lamps. The frame could be moved either by hand or mechanically along with the length of the chamber with a to and fro movement of 20 cm with the help of a device attached to it. On the wall of the chambers above and under the frame are placed ventilators for blowing out the water vapours appearing in the chamber during the process of drying. For maintaining exact temperature on the skin surface contact thermometers were used. The chamber was installed with power meters for recording the current consumed, while on the frame hygrograph was installed for recording the relative humidity during the process of drying.

Experimental drying was done according to the following procedure[3]. The skins were cut along the white line and then cleaned ; hind legs, head and other unwanted parts were removed as well. Skins prepared under the described method, were put upon the frame flesh side facing the lamps with special attention not to permit any folds or overlapping of the skins.

After inserting the skins in the chamber, the lamps were ignited and the ventilators were put in rotation. The temperature on the surface of the skins was 28-30°C which corresponded with 38-39°C in the depth of the skins. To dry sheep skins, containing too much fat, a temperature of 26-27°C was used as otherwise the fat might get melted [4, 5] thus hindering the normal drying process. The relative humidity of the air was maintained at about 35%. During the drying, the frame was moved to and fro at an interval of several minutes to eliminate overheating of certain spots on the skins, since the heat was radiated from separate heating elements. The moment for finishing the process was estalbished by feeling the skin with hand and the dryness of the wool.

The dried skins were taken out of the drying chamber and left freely hanging for 2-3 days. This is compulsory as the skins immediately after being out of the drying chamber retain more moisture than in normal dry skins (15% for sheep and 12.5% for lamb skins). This fact, however, is no handicap for arranging them close to one another as there is no potential possibility for development of a decaying process. After the drying is over, the skins may be put away for storage in the usual manner.

Following the said technological procedure, during the years 1964-65 trials were made with a lot of sheep and with three lots of lamb skins.

The number of skins with the different lots was : sheep—32 with average fresh weight of the skin 3, 021g (from2,350 to 4,130 g), young sheep — 22 with average fresh weight 1,470 g (from 420 to 2,000 g), lambs — 1st lot—39 with average fresh weight 970 g (from 570 to 1,000g), 2nd lot — 51 with average fresh weight 902 g (from 680 to 1,550g), 3rd lot — 64 with average fresh weight 938 g (from 650 to 1,450g). The sheep skins were with full and half wool and the young sheep and lamb skins were with full wool. The length of the wool fibre was within thel imits, sheep 3.5 to 9.5 cm, young sheep 2.5 to 9 cm and the lamb 1 to 8 cm.

The duration of the drying process for the different lots of skins was as follows : for the sheep skins 5 to 8 hr, young sheep skins 4 to 7 hr, lamb skins — 1st lot 3 to 7 hr, 2nd lot 4 to 6 hr and 3rd lot 4 to 7 hr. For the different kinds of skins this length of time was in relation to different factors like, the size and thickness of the skin, the quantity of fat within the skin, the moisture content of the wool, the corvelation between skin and wool and others.

Like natural drying, the removal of the water from the skin under the described technological procedure of drying by infrared rays, is more intensive during the early hours of irradiation, with slower evaporation thereafter[4]. While with natural drying, this water evaporation lasts days and weeks, here it only lasts hours. The fast evaporation during the early hours of radiation includes the superficial and part of the capillary water of the skin, while the inner capillary water is separated and evaporated rather slowly. The retarded evaporation of the inner water makes necessary the stopping of the radiation before the skin has acquired the standard moisture content. Otherwise there will be unnecessary expenditure of electric power and there is always a potential risk for destroying the collagen of the skin derma. Moreover, the moisture content retained by the skin immediately after drying by irradiation is not appreciably higher than that of a perfectly dried skin.

Results and Discussion

Expenditures on electric power for drying the skins by infrared rays are given in Table 1.

It is seen (Table 1) that the expenditure on electric power is greater for 1 kg dry weight of lamb skin, than sheep and young sheep skins. This is explained as due to the relatively greater skin area, and smaller quantity of wool (shorter fibres) in the lamb skins in comparison to sheep skins. Another reason for the greater consumption of electric power is the slightly higher water content of the lamp skins in comparison to the sheep skins.

TABLE I

Electrical power consumption for infrared drying of skins

Kind of skin	Electrical power in KW for 1 skin (limits)	Electrical power in KW for 1 kg dry skin (average)	Cost of electrical power needed in st (1 leva = 100 st)	
			1 skin	1 dry skin per kg.
Sheep	8 —14	6.5	16—28	13
Young sheep	5.5—7	6.5	11—14	13
Lamb	2.5—5	9	5—10	18

Different lots of dried skins were evaluated in raw dried state and during processing. The organoleptical evaluation of the dried skins showed, that they except in certain cases, were well dried with a slight gloss towards the wool side and with no visual harm to the wool itself. On soaking the dry skins for the necessary period in clean water, they showed a gain in weight of more than 315% of dry substance and the skins had a normal fresh look. The water content of the dried skins when put in storage was within the limits of the standard.

For the final evaluation, the dried skins were submitted to factory proces-ing ; the sheep skins, some of the young sheep skins and two lots of lamb skins were processed without wool by chrome tanning, and the rest of the young sheep skins and one lot of lamb skins were processed with wool by the combined method for use in the textile industry. Following the factory processing, it was seen that there was no difference between the experimental dried skins and the controls dried under favourable natural conditions. These experimental and control leathers were compared and evaluated organoleptically and were found comparable to each other.

Data for the physicomechanical indexes are given in Table 2.

It is apparent that there is no great difference between the control and experimental leathers. In general, the infrared dried skins do not appear to result in leathers of inferior qualities (in some cases better indexes).

TABLE 2

Physicomechanical indexes for leathers from sheep and lamb skins

Kind of skin	Type of factory process	Physicomechanical indexes (Average values)							
		Surface cracking (kg/sq mm)		Stretching strength (kg/sq mm)		Load extension %		Bursting extension %	
		E	C	E	C	E	C	E	C
Sheep	Leather	1.00	0.90	1.41	1.27	62	55.2	78.4	65.4
Young sheep	Leather	1.33	0.90	1.76	1.27	40.8	55.2	69.0	65.4
Young sheep	Fur	0.46	0.44	0.58	0.61	—	—	27.4	32.2
Lamb I lot	Leather	0.71	0.77	1.14	1.16	86.0	82.6	106.0	105.8
Lamb II lot	Fur	0.52	0.44	0.70	0.61	33.8	—	38	32.2
Lamb III lot	Leather	0.61	0.45	0.95	0.77	27	22	54	42.8

E—Experimental ; C—Control.

At the end, the cost of drying lamb skins under natural conditions and in drying chambers by infrared rays may be considered. Calculations are made for 1,000 pieces of skins (600 kg dry weight) per day. However, the drying equipments is in full use only for 7 months in a year, during which period the lamb and sheep skins are dried. The quantity of lamb skins produced daily in a middle sized packers plant is about 1,000, which in turn would produce about 140,000 lamb skins (the necessary space for sheep skins is equalised with that for lamb skins) per year. A floor space of 1,500 sq m would be required to dry 1,000 lamb skims daily (based on a 15 days long natural drying) and this would be in two rows vertically. As per the technological process the greater part of the moisture is evaporated during the first days of the drying, and after several days the skins could be accomodated in a smaller floor space. Thus, there will be a reduction of the floor space by about 30%. The duration of the drying by infrared rays is significantly short. For drying 1,000 lamb skins daily, a drying chamber with a total length of the tunnel 150m, width 1.25 m and height 2 m would be needed. For further drying of the skins an additional floor space of about 150 sq.m would be needed.

Capital investment in the drying process by infrared rays is smaller and the turnover of the skins is greater. This permits a regular production and delivery of raw materials to the factories. By using the drying equipment for drying other products of the packers plant and with an eventual lowering of the cost of electric power, the output expenditure with this kind of drying technique will be equal to the expenditure of natural drying process.

From the above observations it can be concluded that infrared rays can be used for drying of raw skins in special drying chambers following the exact prescribed piodecure. The drying of the skins by these rays is 3 to 4 times quicker than the artificial drying by hot air and manyfold quicker than drying under natural conditions. With the present cost of electric power for drying under the said method, the production expenditure will be augmented and the unit production cost will be greater.

A diminished time for drying, a greater turnover of the dried raw materials and their fast expedition to tanneries, are the positive sides of the infrared drying method. Such a drying process could be tried out in real production conditions (in 1 or 2 packers plants) if the cost of electricity could be reduced. The introduction of such a drying method would be easier if the drying chambers equipped with infrared rays units could be used for drying other plant products.

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Centralised Treatment, Storage and Sale of Hides and Skins

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The paper describes the methods adopted in cooperative slaughter houses in Denmark for flaying, trimming, curing and handling of hides and skins. After primary salting, the hides and skins are sent to centralised warehouse of 'Hude Centralen', a cooperative sales organisation, where the hides and skins are treated further, stored for better preservation and are marketed. The part played by 'Hude Centralen' in improving the quality of Danish hides and skins is emphasised.

In spite of the unfavourable climatic conditions and rather barren soil, the Danish agricultural activity plays an important part in the Danish national turn over, and actually, about half of the total Danish export is covered by agricultural products. Until about one hundred years ago, the Danish farming was carried out on an old tradition and was mainly based upon the self-sufficiency system. The farmers used to kill their own animals, flay off the hides and skins and preserve the meat by salting it for their own consumption during the winter. The sheeps were clipped and the wool was treated and spun for weaving of cloth for home made dresses. The only fertiliser used was the dung from the animals. The excess production of grain, home-churned butter, eggs, etc., was delivered to the local tradesman together with the hides and skins in barter for colonial goods and other household utensils.

The other important trade existed at the time was the export of live cattle which were being driven along the roads over the southern border to Germany. Gradually the South- and North-American markets became very serious competitors to the Danish agricultural products and the Danish farmers faced a very serious crisis.

Cooperative organisation : Prominent and farsighted farmers, however, took up the challenge and realised, that only by acknowledging the changed conditions and by rationalising the treatment and the sale of the various products, it was possible to survive. This led to the erection of the cooperative dairies and slaughterhouses, as well as, other cooperative organisations, as buying firms for fertilisers and machinery and sales organisations for bacon, meat, butter, eggs, etc. By these combined efforts, it was possible to increase the output of the harvest and to obtain a more rational and centralised treatment and sale of the products.

By establishing cooperative breeding centres for the best suitable animal races, the Danish farmers have now succeeded in producing high quality products of an even standard, which are appreciated all over the world. This development has been followed up also in the field of hides and skins and has led to the establishment of ' Hude Centralen,' a firm which is the cooperative sales organisation of a large number of slaughterhouses, cattle-buying and exporting organisations, for selling the hides and skins.

Nearly all the slaughtering in Denmark takes place in public or cooperative slaughterhouses, as the veterinary rules are rather strict and make it nearly impossible to slaughter privately.

'Hude Centralen' handles about fifty percent of the total Danish production while the remaining half is being handled by a number of private enterprises. It has two warehouses, one situated in Glostrup and one in Brabrand. They are nearly of the same size and are provided with refrigerated cellars and possess adequate facilities for storing the hides and skins in ideal conditions.

Handling and curing of hides : After flaying, the hide is removed to the hide cellar or hide room, which is usually placed below the slaughtering room. It is important that the hide room is maintained at a low temperature (around 7 to 8°C) and the hide is to be cooled down sufficiently before being salted. The best way to cool the hide down is to spread it out in the hide room and let it rest for some hours. Superficial cooling or washing with cold water may be rather harmful as only the outer part of the hide is cooled down, the middle part remaining still warm. This may lead to the formation of salt stains and may cause deterioration during the comparatively slow salting process. Before being salted the hides are trimmed in accordance with the rules of trimming Danish hides and skins.

When the hide is cool, it is spread out carefully and salted with 35 to 50% salt on the weight of the green hide. Only clean and new rock salt (number 2)

must be used. The salt must be as close to 100% pure, as one can get, but the contents of calcium sulfate must not be more than 1%. Ground salt finer than number 2 should not be used, as later on, when the hides are weighed in salted state, it is difficult to remove them. Granular salt, 100% pure and if available at reasonable price is recommended. The salt should only be used once, as the preserving ability is too low in used salt.

The whole hide must be covered well with salt especially the head and the legs. During the salting process, the hides must be piled, stretched out on top of each other, allowing blood and moisture to drain out well.

Each hide is provided with a wooden tag, stating the number of the slaughterhouse and a consecutive number. The hides are weighed and the weight is written on a list in the respective column, for instance, heifer hides-/24 kilos. Further remarks needed, as for instance, holes, cuts etc. are written in a special column. Due to this system, it is always possible to trace back the respective slaughterhouse from which the hide has been obtained and often the butcher who has flayed the hide.

The hides are piled under the first salt for 4 to 5 days and then bundled. If the original salt has been used up, some extra salt is used in making the bundles, and are sent by rail, truck or ship to the warehouses.

Treatment in warehouses : After receiving the hides in the warehouses they are assorted, weighed, graded for quality and compared with the lists obtained from the slaughterhouse. They are then salted again only with new, clean salt, and kept in big piles. Separate piles are made for each range of hides, so that they can be taken out easily when they are to be shipped.

The hides are kept in piles under salt at an artificial temperature of about 8°C and can be preserved for a considerable period without getting any preservation defects.

It is not wise to further lower the temperature, as this is unnecessary, uneconomical and even dangerous. When the hides are taken out in a hot summer (25-30°C) from a very low temperature and are exposed or transported, they are subjected to fast deterioration.

This second salting of the hides is very important, as it produces hides of uniform quality. Salted in the same way and together with the standard assortment in two similar warehouses, the buyer is always sure to get the same goods from time to time and is able to calculate exactly how much leather he gets from these hides.

When the hides are sold, they are taken out of the piles, shaken free from excess salt, bundled, weighed and sent by truck, rail or ship.

Ten to eleven times a year, calculations are made to find out the sales price for each of the various ranges of hides and skins.

Deducting the handling costs, the prices that are to be paid to the slaughterhouses for the coming period are decided. When the financial year is over the profit is paid back after deducting reserves, depreciation, provisions, etc. This paying back is mainly done on the basis of per kilo delivered green weight from each slaughterhouse.

Marketing : There are various methods of selling hides and skins and in some European countries the hides are sold on auctions. 'Hude Centralen' sells the hides directly in the free market all over the world and finds this to be the most profitable way of marketing. About 75% of the hides that are dealt with by 'Hude Centralen' are exported and with the ever changing conditions of the market efforts are always being made to find out newer markets where goods can be sold advantageously. To achieve this and to have personal contact with the buyers visits to the customers are arranged quite often.

The Danish hide is suitable for upper leather of a very high quality and also for upholstery purposes and as the quality of the hides is maintained to a very high order it has not been difficult to market them. The sale of hides and skins in Denmark is based upon the "Copenhagen Agreement" which has been accepted by buyers and sellers all over the world. In this agreement, with supplements and amendments, rules are laid down for the assortment of so called hammerflayed hides and ordinary hides, while machine flayed hides are not mentioned, as they were not produced at the time this agreement was made.

In Denmark 'Hude Centralen' is the only organisation handling hammerflayed hides in sizable numbers. These are charged with an extra 5% and are provided with special plastic tags.

Rules are also framed under the "Copenhagen Agreement" as to how the goods must be shipped and weighed, as well as, the franchises to and between various harbours.

The pattern for trimming the hide has been changed several times and this organisation has taken very active part in preparing these changes. Well

trimmed hides now produced according to the 'Scandinavian trim' pattern is saving the amount of salt used for curing, freight charges and subsequent trimming in the tanneries. It is a pleasure to see that countries like USA have adopted this method of trimming.

Improvement of hide quality : One of the objectives of ' Hude Centralen ' is the hide improvement work. If something goes wrong with the flaying or preservation in any slaughter house advice of the centre is sought of to improve on the quality.

Hide improvement work is very difficult, as the hide is considered by-product, and when compared with the price of meat, hide has got much less value.

The competition is very hard today and every penny counts. So the centre is going ahead with the propaganda, which has two fronts :

1. Natural defects on the living animal
2. Flaying and treatment defects.

Some years ago Denmark had a great problem with the warble fly, but through a very intensive campaign in which the veterinarians took a very important part, it was possible to succeed in exterminating this defect, and today the warble fly does not exist in Denmark.

This was a very inspiring example of what can be obtained by rational information and action. ' Hude Centralen ' centered the propaganda upon impressing the fact that

' A SOUND WELL KEPT ANIMAL GIVES MORE MILK AND MEAT '

Everybody can understand that a cow, troubled by sores, warts, heavy dung on the flanks, ring worms etc. falls sick very easily, and thrives badly and it will take longer time for the animal to be fit again which means more feeding stuff and less milk. Animals affected in either way gives less money to the farmer and that is an argument a farmer understands. ' Hude Centralen ' has got a film with which they go to various markets and assemblies of farmers and show them the hide defects, as well as, how to overcome these defects.

With regard to the eradication of flaying and other defects, proper advice on improved techniques is offered to individual butchers from time to time. Attempts are being made for more machine flayed hides, which make cuts

and scores less frequent. Proper training in modern techniques of flaying hides and skins is imparted to young apprentices and incentives are offered if they are capable of producing a perfectly flayed hide in their final test.

The main prescribed rules for the treatment of hides and skins in the slaughterhouses are as follows :

(*a*) Careful ripping and take off, omitting wrong pattern cuts, scores and holes

(*b*) Tidiness in handling of the hides

(*c*) Careful cooling and draining after the slaughtering

(*d*) Plenty of clean salt spread out well over the whole hide

(*e*) The salting room must be kept cold, preferably at 7 or 8°C

It has been experienced that more preservation defects originate from the treatment of hide in fresh state and by improper primary salting and mainly because they are salted in a too warm state. The conditions in Denmark with regard to hides and skins are not too bad, but there are many improvements yet to be hoped for. The objective of ' Hude Centralen ' is to concentrate cattle slaughter in 12 to 15 slaughterhouses, each with a yearly output of 75,000 to 1,00,000 heads.

If this becomes true one day, the whole procedure could be changed to centralised brine curing after defleshing and demanuring of the hides as they do in many places in USA today.

This method is not adoptable in Denmark due to the scattered slaughtering of animals in many slaughterhouses. During later years, better substitutes for leather have been made and some of them appear to be very close to the real leather and it is certain that the cheaper qualities of leather will find a very hard competitor, in substitutes. Badly flayed and treated hides and skins may not be worthwhile tanning because of the high cost of production.

The finer qualities of leather will, in spite of price, always find buyers, who want the luxury of real leather and there will always be a market for the high quality shoes, bags and upholstery. Therefore, it is very important for the farmers, hide dealers and tanners to realise that hide improvement is most essential to produce better quality finished leathers.

Change in Properties of Raw Hide and Skin during their Preservation and Beamhouse Operation

Part II

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This experiment was carried out to elucidate the effect of chlorotetracyline in curing hides and skins. The influences of different storage conditions on the distribution of nitrogenous components in cured skins and on the properties of collagen were compared. The results obtained indicated that during storage of green salted skins the quantities of non-protein nitrogen and the amounts of collagen solubilised by alkali were increased but the quantity of amide nitrogen in collagen was decreased. When staled skin was salted and stored in 80% RH at 25-28°C after being kept for 2 months at 5-8°C, these changes were remarkably increased. But soaking of the skin in 10% NaCl solution containing 20 ppm chlorotetracyline prior to curing appreciably reduced such changes. These results indicate that chlorotetracycline is useful as a disinfectant in curing.

Raw hide whose freshness has dropped to some extent due to staling can be preserved after salting without any further deterioration in quality by soaking it with a 10% NaCl solution to which 20 ppm of chlorotetracycline has been added[1]. In view of this, the change in the composition of nitrogenous components in the salted skin treated with chlorotetracycline when stored at high humidity and also at a relatively high temperature, and the change in the properties of collagen obtained from such salted skin were studied.

Materials and Methods

Two skins (7 lbs) collected from cattles raised in the suburbs of Kanto area in Japan, were used. After fleshing, the skins were washed for 10 min with cold water and the excess water was drained out and cut into two equal parts at the back line IA, IB and IIA, IIB from each. IA was soaked for 16 hr in 5 times its weight of 10% NaCl (20 ppm of chlorotetracycline added) solution. Temperature was maintained at 14-17°C. After soaking it was horsed up to drain out water, and then salted with 3 times its weight of industrial salt (purity above 95%, 50 mesh) and kept in a wooden box. IB was salted in the same manner as IA with the exception that addition of chlorotetracycline was omitted. IIA and IIB were staled for 3 days in the room temperature at 25°C and at 67% RH and then treated in a similar manner as in the case of IA and IB respectively. The skins were piled for 40 days.

Method of salting after addition of salt : After piling for 40 days, 8 samples were obtained from the back portion as shown in Fig. 1. Specimens 1-4 obtained from each salted side were left at a constant temperature of 25-28°C and 80% RH, while specimens 5-8 were left in a refrigerator at 5-8°C for 2 months and then preserved in the aforementioned constant temperature of 25-28°C and 80% RH for 1 month, 2 months and 4 months, and these were removed as required and used in the experiment.

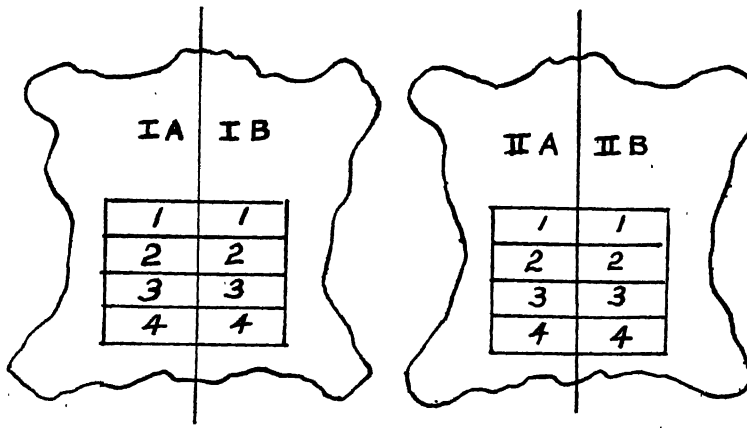

Fig. 1. *Diagram of the hides showing sampling positions*

Fractionation and quantitative analysis of nitrogenous components : Nitrogenous components of salted specimens were determined by the method used previously[2].

Hair was scraped off completely from the rest of the specimen used for fractionation and quantitative analysis of nitrogenous components and soaked in 5 times its weight of 10% NaCl solution (NaCl in the skin included in the weight) for 48 hr at 2-5°C. During this, the soaking solution was renewed every 12 hr. After completion of soaking, it was washed in a small test drum and defatting and dehydration were carried out with acetone. Then, it was air-dried. The middle layer was obtained by splitting the specimen parallel to the grain surface and discarding the grain layer and flesh layer. It was cut into small pieces and pulverized in a willey mill. This powdered skin was soaked further in 10% NaCl and 10% $CaCl_2$ solution with *p*H adjusted to 8.0. After deliming it was washed with water and dehydrated with acetone[3]. The powdered skin obtained by the above treatment was taken as the refined collagen and the amount of amide nitrogen, absorption of chromium perchlorate and trioxalate chromiate at *p*H 3.8 and 2.2 were measured. In order to compare the eluted quantity of nitrogen with that of liming, 2 g of collagen was taken and soaked for 2 days at 20°C in 50 ml of saturated lime water, the eluted nitrogen was determined by the Kjeldahl method and the percentage against total nitrogen of collagen was obtained and taken as the quantity of eluted collagen[4].

Results and Discussion

The change in composition of nitrogenous components in skin IA, IB and IIA, IIB is shown in Table 1. IA contained 2.20% of nonprotein nitrogenous components immediately after salting and increased slightly to 2.44% after 4 months. However, the change is very small when compared with the increase from 2.27% to 3.34% for IB.

The non-protein nitrogenous component of IIA and IIB immediately after salt addition are 2.67% and 3.45% respectively, and are more than IA and IB. After salting for 4 months they increase to 3.29% and 4.95% respectively. Consequently, the effect of chlorotetracycline is clearly observed by the extent of increase of nonprotein nitrogenous component and the effect is particularly marked in case of staled skin. The quality of salted skin became poor rapidly when it was first stored in a refrigerator and then stored at high temperature and humidity[5]. With regard to this point, the effect of chlorotetracycline has been investigated.

TABLE 1

Changes in composition of nitrogenous components in skin stored in 80% RH and at 25-28°C

Samples	Nitrogenous components	Period of storage (Months)			
		Initial	1	2	4
IA	N.P.N.	2.02	2.37	2.29	2.44
	S.P.N.	6.91	6.72	6.84	6.63
	L.S.N.	2.92	2.90	2.87	2.81
	C.N.	88.15	88.01	88.00	88.12
IB	N.P.N.	2.27	2.89	3.27	3.34
	S.P.N.	6.86	6.64	6.35	6.47
	L.S.N.	2.86	2.90	2.81	2.79
	C.N.	88.01	87.57	87.57	87.10
IIA	N.P.N.	2.67	2.87	3.01	3.29
	S.P.N.	6.42	6.27	6.33	5.99
	L.S.N.	2.86	2.90	2.88	2.80
	C.N.	88.05	87.86	87.78	87.89
IIB	N.P.N.	3.49	3.92	4.01	4.59
	S.P.N.	5.89	5.72	5.64	5.54
	L.S.N.	2.84	2.88	2.71	2.80
	C.N.	87.78	87.44	87.64	87.07

(Calculated as percent against total nitrogen of skin except elastin-keratin nitrogen)

N.P.N : Non-protein nitrogenous component
S.P.N : Soluble protein nitrogenous component
L.S.N : Lime soluble nitrogenous component
C.N : Collagen nitrogenous component

The skins were stored for 2 months at 5-8°C and then preserved for 1, 2 and 4 months under the aforementioned condition (80% RH, 25-28°C). The composition of the nitrogenous components in the salted hides is shown in Table 2.

TABLE 2

Change in composition of nitrogenous components in skin stored in 80% RH and at 25-28°C after keeping for 2 months at 5-8°C

Samples	Nitrogenous components	Period of storage (Months)			
		Initial	1	2	4
IA	N.P.N.	1.94	2.24	2.17	2.39
	S.P.N.	6.89	6.92	6.82	6.75
	L.S.N.	2.89	2.84	2.91	2.80
	C.N.	88.28	88.00	88.10	88.06
IB	N.P.N.	2.29	3.20	3.46	3.94
	S.P.N.	6.79	6.72	6.47	6.31
	L.S.N.	2.80	2.83	2.80	2.77
	C.N.	88.12	88.45	88.27	86.88
IIA	N.P.N.	2.72	2.92	3.36	3.42
	S.P.N.	6.40	6.32	6.29	6.22
	L.S.N.	2.79	2.83	2.81	2.76
	C.N.	88.09	87.93	87.54	87.60
IIB	N.P.N.	3.55	4.03	4.73	5.37
	S.P.N.	5.74	5.65	5.42	5.26
	L.S.N.	2.80	2.74	2.70	7.79
	C.N.	87.91	87.48	87.12	86.65

(Calculated as percent against total nitrogen of skin except elastin-keratin nitrogen)

N.P.N : Non-protein nitrogenous component
S.P.N : Soluble protein nitrogenous component
L.S.N : Lime soluble nitrogenous component
C.N : Collagen nitrogenous component

The content of nonprotein nitrogenous component increases in IA, IB, IIA and IIB but the increase in IA and IIA is very small when compared with the increase in IB and IIB, and the difference is particularly great in the case of staled skin (IIA, IIB). That is, it is possible to maintain the quality in a comparatively good conidtion by the addition of chlorotetracycline even in case it is preserved under unsuitable conditions. The amide nitrogen of collagen, quantity of chromium absorbed and quantity of alkali elute from the various salted skins which were stored at 25-28°C and 80% RH and others which were kept in a refrigerator and then stored under similar conditions are shown in Tables 3 and 4.

TABLE 3

Changes in characteristics of collagen in salted skin stored in 80% RH and at 25-28°C

Determinations	Samples	Period of storage (Months)			
		Initial	1	2	4
Amide Nitrogen	IA	2.47	2.56	2.63	2.64
	IB	1.68	2.46	2.53	2.44
	IIA	2.52	2.47	2.30	2.22
	IIB	2.43	2.39	2.11	2.07
Absorption of Chromium perchlorate	IA	3.72	3.84	3.69	3.79
	IB	3.66	3.74	3.86	3.82
	IIA	4.02	4.23	4.11	4.30
	IIB	4.29	4.49	4.66	4.93
Absorption of Trioxalate chromiate	IA	2.21	2.14	2.31	2.22
	IB	2.29	2.16	2.27	2.29
	IIA	2.33	2.22	2.35	2.24
	IIB	2.29	2.37	2.44	2.32
The amounts of collagen solubilised by lime water	IA	3.36	3.32	2.40	3.51
	IB	3.26	3.35	3.67	4.27
	IIA	4.03	4.29	4.27	4.62
	IIB	5.43	5.92	6.34	6.73

TABLE 4

Changes in characteristics of collagen in salted skin stored in 80% RH and at 25-28°C after keeping for 2 months at 5-8°C

Determinations	Samples	Period of Storage (Months)			
		Initial	1	2	4
Amide Nitrogen	IA	2.57	2.64	2.62	2.59
	IB	2.50	2.43	2.41	2.39
	IIA	2.36	2.29	2.11	2.07
	IIB	2.21	2.11	2.02	1.90
Absorption of Chromium perchlorate	IA	3.78	3.84	3.76	3.88
	IB	3.72	3.89	3.84	3.80
	IIA	4.22	4.39	4.27	4.22
	IIB	4.30	4.38	4.75	4.89
Absorption of Trioxalate chromiate	IA	2.14	2.21	2.28	2.22
	IB	2.22	2.17	2.14	2.25
	IIA	2.26	2.32	2.41	2.27
	IIB	2.29	2.34	2.34	2.30
The amounts of collagen solubilised by lime water	IA	3.67	3.71	3.57	3.80
	IB	3.43	3.57	3.89	4.37
	IIA	4.11	4.46	4.79	4.99
	IIB	5.63	6.02	6.61	7.42

Compared with IA and IB, the content of amide nitrogen is smaller in case of IIA and IIB, and that of IIB becomes very small when preserved for a period of 4 months after 40 days salting (from 2.43% to 2.07% after 4 months). In IIB the absorption of chromium perchlorate is more than the reduction in amide nitrogen content. No difference is noted in the quantity of trioxalate chromiate absorbed. Consequently, it is believed that staling and salting did not affect the ε-amino radical of collagen which is considered to be participating in this absorption. The elution quantity of nitrogen by lime water increases in IA, IB, IIA and IIB by salting but the increase is particularly large in case of IIB. That is, the nitrogen content of 5.43% after 40 days salting has

increased to 6.73% after storing for 4 months. While comparing Tables 3 and 4, the changes in the reduction of amide nitrogen, absorption quantity of chromium perchlorate and quantity of nitrogen eluted by lime water appears to be more in case of skins which are kept at 5-8°C for two months prior to storage in 80% RH and at 25-28°C. Thus, it is believed that the changes become marked when skins are first refrigerated and then stored at high temperature and humidity.

In summarising the above results, it has been made clear that the extent of change in the properties of collagen is smaller when chlorotetracycline is added during salt-curing than in case it is not added.

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Quality Index for Cattle Hides for Upper Leather—a Suggested Method

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Investigation was carried out to find out a method by which the quality of raw hides for the manufacture of upper leather could be assessed. Tightness of the grain and fulness of the leather were found to be influenced to a considerable extent by the cumulative effect of (i) natural tightness of the grain, (ii) merging of the grain into the corium and (iii) compactness of the fibre texture. The first two factors were measured directly and the third one indirectly. A method for numerical assessment of cattle hides based on these factors has been suggested by which the quality of the hide can be determined with some degree of accuracy.

Very little work has been reported regarding quality index for raw hides. The lack of literature on this subject may be due to the fact that the variations in the structure of hide are so great that it is often difficult to measure the exact differences between any two hides in any particular tanning process. The major problem which often causes much worry to tanners is to determine from visual examination whether the hide will produce a tight or loose grained leather with a soft and mellow feel or a hard leather with a papery feel. Practically there is no particular manufacturing process by which tight-grained and firm structured leather could be guaranteed. The tightness of the grain and softness of the feel with good temper, are the most outsanding charactereristics of quality upper leather made from cattle hides. The smoothness of the grain surface is, however, specially required in the case of leather made from skins. Many factors influence these properties and some of them are beyond the control of the tanner. This investigation does not, however, minimise the importance of other technological aspects of leather tanning and finishing which greatly contribute to tightening of the grain layer. It is the object of this study to emphasise that the many types of skin structure corresponding to the various species, age and other background factors of the raw material explain the reasons for differences in grain tightness from hide to hide although they have been subjected to the same

chemical and mechanical treatments. Hence, the problem starts with the raw materials. The tightness of the grain and soft and mellow feel of the leather depend to a considerable extent on the inherent characteristics of the raw hide. So this work was undertaken with a view to find out a method by which the quality ot raw hides for the manufacture of upper leather could be assessed in the shortest possible time with some degree of accuracy.

Materials and Methods

Three grades of cat'le hides each consisting of 10 representative hides were taken. Group 'A' consists of hides (Nos. 1 to 10) with very good substance, group 'B' (Nos. 11 to 20) with medium substance and group 'C' (Nos. 21 to 30) with poor substance. All the hides were free from defects. Cut pieces of samples, each measuring $3'' \times 2''$, were taken from butt and belly regions. The exact position of the pieces taken is indicated in Fig. 1. The remaining portions of the hides were tanned and finished as box sides following a standard method. Again small cut pieces, each measuring about 1 inch square, were taken from the cut samples for sectioning with freezing microtome. The pieces were soaked in water to bring them back to normal condition. Instead of fixing the pieces in the usual 10% formol saline for 48 hr, frozen sections were cut from the soaked pieces and collected in 3% formol saline. After 15 minutes, the sections were washed in distilled water and mounted in glycerine jelly. Merging of the grain into the corium was examined in all the samples and the data are shown in Table 1. The remaining portions of the cut samples, were freed from adhering salt as far as possible and were used for the determination of moisture content, water-uptake and natural grain-tightness. Moisture content of the sample was determined by following the official method[1]. For water absorption, pieces measuring $1\frac{1}{2}$ inch square were taken from the remaining portions of the cut samples and water uptake was measured by means of Kubelka apparatus at intervals of $\frac{1}{2}$ hr upto a maximum period of 3 hr. Water uptake for $2\frac{1}{2}$ hr is recorded in Table 1.

The natural tightness of the grain was measured by determining the percentage shrinkage of the area of the grain in relation to that of corium in wet condition of the hide after removing the grain layer by split'ing in a manner similar to that described by White and Caughley[2] wtih certain modifications. This measurement was made with wetsalted hides after soaking back in water. The exact thickness of the grain layer of the hide under study was first determined from the vertical section. Then a small piece of about 3 inch square was taken from the cut samples, hair shaved off as far as possible, and the piece soaked and washed in running tap water until it regained its natural

condition. The grain layer was then cut horizontally and split off with the freezing microtome to its predetermined full thickness, and the split grain and corium collected in water. The split grain was then floated on a small clean glass plate and allowed to spread out with the grain surface downward.

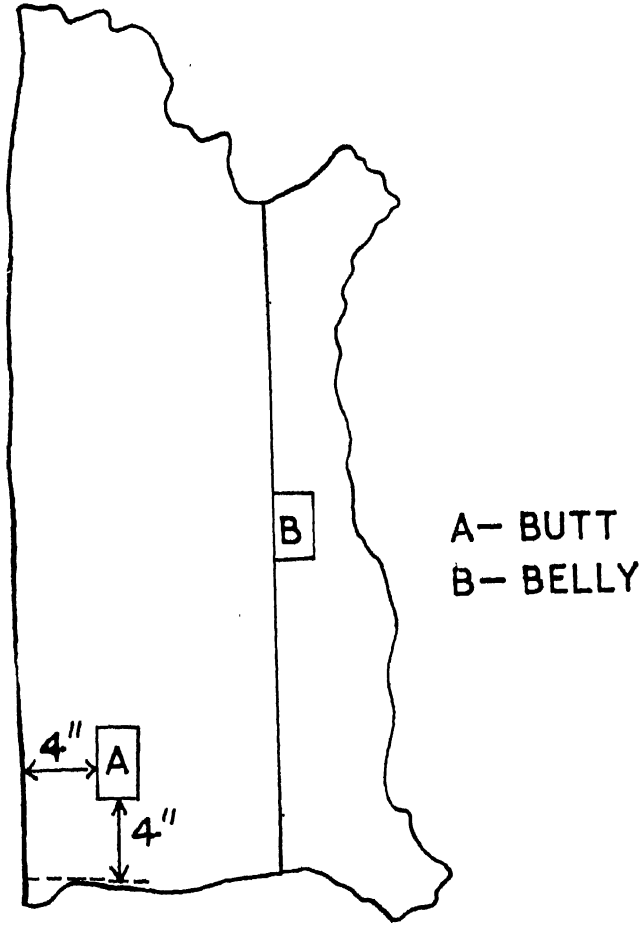

Fig. 1. *Diagram of side of a hide showing sampling positions.*

Excess of water was drained off and the adhering water from the edges of the fully spread out grain layer removed carefully with blotting paper. On the underside of the glass plate, the boundary line of the split grain was marked with Indian ink and the outline traced on a tissue paper. The area of the split grain was then found out by measuring the area enlcosed by the outline, with a planimeter. The area of the split corium was measured in the same way, and the percentage shrinkage of the area of the split grain was determmined.

Results and Discussion

Natural grain tightness of the samples are given in Table 1.

TABLE 1

Natural grain tightness, merging of grain into corium and water absorption of raw hides.

Hide No.	% Natural grain Tightness		Merging of grain into corium		% water absorbed in 2½ hr.	
	Butt	Belly	Butt	Belly	Butt	Belly
1	18.1	15.9	F.G.	F.G.	47.06	81.11
2	31.3	22.6	F.G.	F.G.	37.34	48.91
3	18.8	12.6	G.	F.G.	31.11	42.75
4	16.4	12.0	F.G.	S.P.	56.04	58.82
5	18.8	17.0	S.P.	S.P.	38.88	52.27
6	18.4	11.5	F.	F.	39.75	56.80
7	9.1	8.7	P.	P.	52.13	67.81
8	16.9	9.6	P.	S.P.	51.24	61.81
9	16.4	12.8	F.G	G.	47.55	57.33
10	21.6	16.4	F.	F.	41.26	54.30
11	13.7	10.8	F.	P.	51.05	60.62
12	14.0	11.7	F.G.	F.G.	50.53	58.27
13	17.3	14.6	F.	F.G.	56.38	66.37
14	12.1	10.4	F.	F.	49.17	59.15
15	12.3	8.7	V.G.	F.G.	49.38	62.87
16	13.8	7.6	F.	S.P.	58.27	64.11
17	15.4	10.5	P.	F.	55.33	63.15
18	17.8	12.7	F.G.	P.	48.24	50.06
19	12.6	8.3	F.	F.	55.83	58.23
20	16.6	14.7	F.	F.	66.21	69.83
21	12.9	8.1	G.	F.	58.24	64.06
22	17.3	10.3	F.	F.	59.84	68.10
23	11.5	8.6	P.	P.	60.78	69.25
24	9.8	8.5	F.	F.	63.27	66.58
25	9.8	8.4	F.	F.	64.15	71.56
26	16.6	12.6	F.G.	F.	48.70	49.75
27	12.9	9.3	F.G.	F.G.	67.42	72.16
28	13.4	10.1	F.	P.	58.62	67.25
29	10.7	8.2	P.	P.	62.23	68.21
30	13.7	10.3	F.	F.	62.77	68.17

G.-Good; F.G. - Fairly Good; V.G. - Very Good; F - Fair; P - Poor; S.P. - Slightly poor.

The physical characteristics of the final leather particularlly, feel, fulness, grain tightness and fineness of the break were assessed and recorded in Table 2.

TABLE 2

Physical characteristics of finished leathers

	BUTT				BELLY			
Code No.	Tightness	Break	Feel	Fullness	Tightness	Break	Feel	Fullness
1	T	F	Soft, Mellow	Full	T	F.F.	Very soft	Empty
2	V.T.	F	Soft, Temper	Full	T	F	Soft, mellow	Full
3	T	F	Soft, Temper	Full	F.T.	M	Soft, Mellow	Full
4	T	F.F.	Soft	F.Full	S.L.	S.C.	Very soft	F.Full
5	F.T.	M	Soft, Temper	Full	F.T.	M	Soft	F.Full
6	F.T.	M	Soft, Temper	Full	S.L.	M.	Very soft	S. Empty
7	L	C	Soft	F.Full	V.L.	C	Raggy	Empty
8	S.L.	S.C.	Soft	F.Full	L.	C	Very soft	S. Empty
9	T	F	Soft, Mellow	F.Full	F.T.	F.F.	Very soft	S. Empty
10	T	F.F.	Soft, Mellow	F.Full	F.T.	M	Soft	F.Full
11	S.L.	S.C.	Soft, Mellow	F.Full	L	C	Very soft	S. Empty
12	F.T.	F.F.	Soft	F.Full	F.T.	F.F.	Very soft	S. Empty
13	F.T.	F.F.	Very soft	S. Empty	F.T.	M	S. Raggy	Empty
14	L	C	Soft, Mellow	F.Full	L	C	S. Raggy	S. Empty
15	T	F	Soft, Mellow	F. Full	F.T.	S.C.	S. Raggy	Empty
16	F.T.	M	Very soft	S. Empty	L	C	S. Raggy	Empty
17	S.L.	C	Very soft	S. Empty	S.L.	C	S. Raggy	S. Empty
18	T	F.F.	Soft, Mellow	F.Full	F.T.	F.F.	Soft, Mellow	Full
19	S.L.	S.C.	Soft	F.Full	L	C	Very soft	Empty
20	F.T.	F.F.	Very soft	S. Empty	S.L.	S.C.	Raggy	Empty
21	F.T.	F.F.	Vert soft	S.Empty	L.	C	S. Raggy	Empty
22	F.T.	F.F.	Very soft	S. Empty	S.L.	M	Raggy	Empty
23	L	C	Raggy	Empty	V.L.	C	Raggy	Empty
24	L	C	Raggy	Empty	L	C	Raggy	Empty
25	V.L.	C	S.Raggy	S. Empty	L	C	Raggy	Empty
26	T	F	Soft, Mellow	Full	F.T.	F.F.	Soft, Mellow	S. Empty
27	F.T.	M	S. Raggy	S. Empty	S.L.	S.C.	Raggy	Empty
28	S.L.	S.C.	Very soft	S. Empty	L	C	Raggy	Empty
29	L	C	S. Raggy	Empty	V.L.	C	Raggy	Empty
30	S.L.	M	Raggy	Empty	L	C	Raggy	Empty

T — Tight; F.T.— Fairly tight; V.T.— Very tight; L — Loose; S.L.— Slightly loose; V.L.— Very loose; F — Fine; F.F.— Fairly fine; M — Medium; C — Coarse; S.C. — Slightly coarse; S. Raggy — Slightly raggy; S. Empty — Slightly empty; F. Full — Fairly full.

On the basis of tightness of the grain and fineness of the break (Table 2), leathers which go under tight grain and fine break in the butt area are Nos. 1, 2, 3, 4, 9 and 10 in group ' A ', Nos. 15 and 18 in group ' B ' and No. 26 in group ' C '. It may be seen from Table 1, that these hides in the butt are characterised by higher natural grain tightness and good merging of the grain into the corium. Although the natural grain tightness in cattle hides varies from 7% to 31%, here it does not fall below 16% except in hide No. 15, and the grain is fairly well merged in most of these hides. The hides numbered 10 and 15 appear to be exceptions in this respect. Although the grain merging is somewhat poor in hide No. 10 it has produced a tight-grained leather. Similarly, in hide No. 15, the natural grain tightness is quite low (below 16%) but the leather made from it shows tight grain with fine break. This anomaly may be explained by the fact that in the above two hides, one or the other of the two conditions viz., natural grain tightness and merging of the grain into the corium, is so well pronounced that one overruns the effect of the other condition.

According to Table 2, the butt of the following hides viz., Nos. 7 and 8 in group ' A ', Nos. 11, 14, 17, and 19 in group ' B ', and Nos. 23, 24, 25, 28, 29 and 30 in group ' C ', indicates loose grain with coarses break. It may be observed from Table 1, that the natural grain tightness in the butt of these hides is quite low and in no case exceeds 16 % except in hide No. 8. Moreover, the grain-merging into the corium in these hides is quite poor. Hide No. 8, however, in spite of fairly high natural grain tightness (more than 16%) has produced a slightly loose leather. This is evidently because the grain layer in this hide is so poorly merged into the corium that the adverse effect of grain-merging overruns the effect of natural grain tightness resulting in a slightly loose grain with a coarse break.

Now, the hides which stand in between tight and loose grain, and fine and coarse break in the butt are Nos. 5 and 6 in group ' A ', 12, 13, 16 and 20 in group ' B ', and 21, 22 and 27 in group ' C '. A comparison of the data in Table 1 and 2 indicate that these hides are characterised by conditions one of which goes in favour of tight grain and fine break, while the other for loose grain and coarse break. It will be evident from Table 1 that the favourable condition for tight grain and fine break in hide Nos. 5, 6, 13, 20 and 22 is the natural grain tightness, but the grain merging into the corium in these hides

is poor which goes in favour of loose grain with coarse break. Reverse is the case with hides numbered 12, 21 and 27. The hide, 16 may be considered as an exception in this respect. The hide, in the butt, having natural grain tightness and grain merging in favour of loose grain and coarse break, has produced fairly tight grained leather with medium to fine break. No suitable explanation is available at the moment for this anomaly.

It will be evident from Tables 1 and 2, that similar conditions exist in the belly region also. The hides having fairly good grain-merging with a fairly high natural grain tightness (16% and above) have produced tight grain with a fine break, and the hides characterised by low natural grain tightness and poor merging of the grain have produced loose-grained leather with a coarse break. Exceptions in this respect are hides numbered 18 and 26. Although both the natural grain tightness and grain-merging in these hides are indicative of loose grain with coarse break, they have produced fairly tight grained leather. No explanation could be offered at the moment for this anomaly. But it will be observed that the butt portion of all these hides having favourable conditions have produced tight-grained leather with fine break. A reference to Table 1 will show that in these two hides the fibre texture as indicated by water uptake is compact both in the belly and butt regions. It may be reasonable to assume that compact packing of collagen fibres has some influence on the tightness of the grain. This is in conformity with the earlier observation[3].

From the above results, it may be concluded with some degree of accuracy that the natural tightness of the grain and the merging of the grain into the corium are the primary factors responsible for producing tight grain with a fine break. The mechanism of these two conditions in the formation of wrinkles (tight or loose grain) can be explained in the following way. When a homogeneous flat elastic substance is folded or bent the outside surface elongates and the inner surface contracts whereby the outside surface becomes longer and the inner surface becomes shorter than the original length. Similarly when the leather is folded or bent with the grain inward, the grain layer is compressed. If the grain is in a state of high tension (expressed as grain tightness in Table 1), the tension is released by folding and the grain contracts automatically and bends without any formation of wrinkles. (Fig. 2)

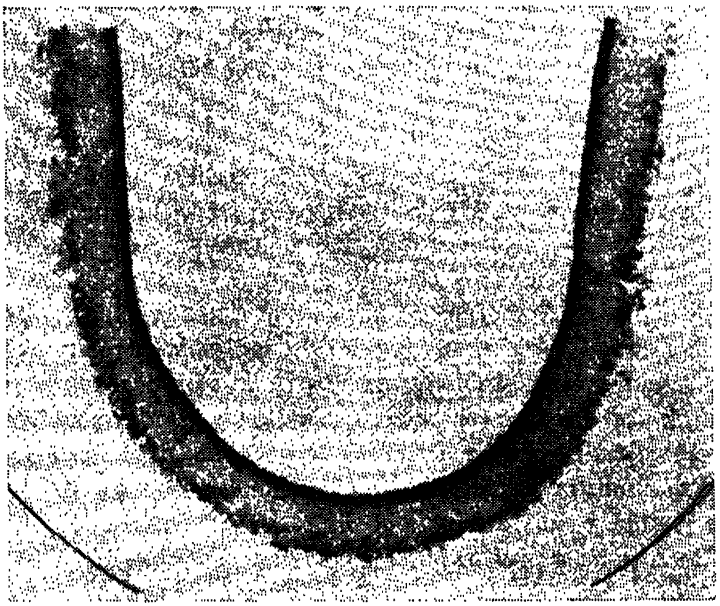

Fig. 2. *Section of a leather having high natural grain tightness in bent condition* (× 6.5)

But, when the tension is very low, then by bending, the grain is compressed beyond a stage where the fibres of the grain layer simply change their relative position and shape, and adapt themselves to a new position with the formation of wrinkles (Figs. 3a & 3b). This produces a condition for coarse break.

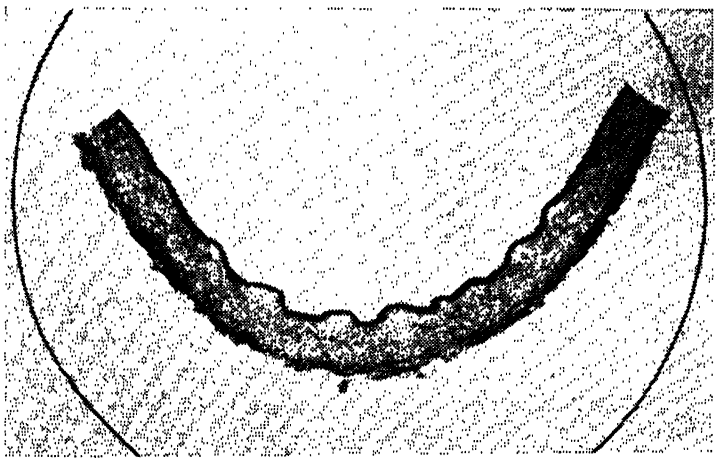

Fig. 3a. *Section of a leather having low natural grain tightness showing formation o wrinkless* (× 5.2)

It will be noticed that where the wrinkles have formed, the grain fibres are detached from the corium and adopt a new shape and position (Fig. 3*b*).

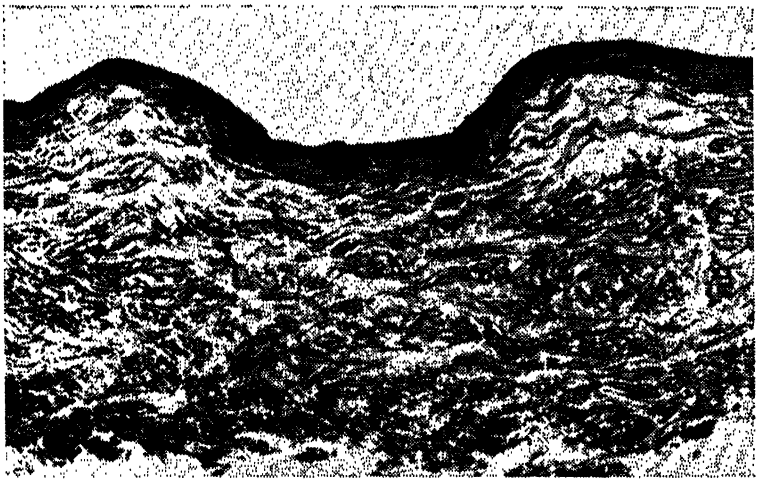

Fig. 3b. *The same leather as in Fig.* 3*a. at higher magnification showing the displacement of grain fibres from the corium at the places of wrinkles* (× 25.8)

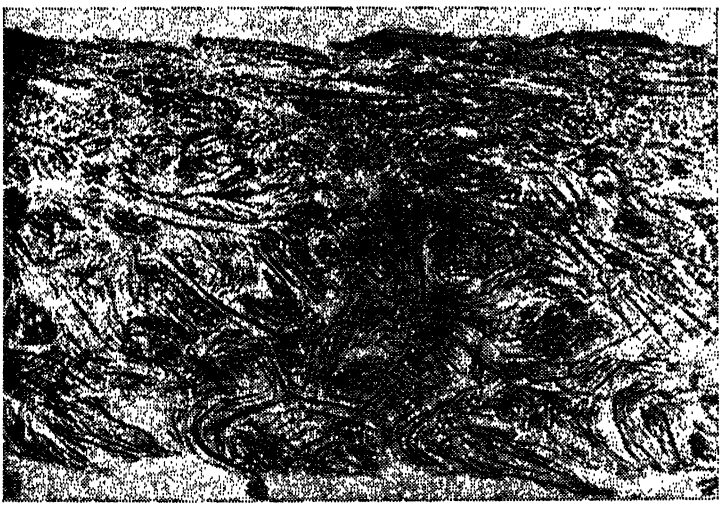

Fig. 4a. *Section of a leather (before dyeing) showing uniform merging of the grain into the corium* (× 36).

Again, when the corium-fibres have firm attachment with grain-fibres (expressed as merging of the grain into the corium in Table 1), it is easier for the grain fibres to be compressed than to change their relative shape and position. When this condition of fibres exists, the leather forms fine break on folding (Figs. 4*a* and 4*b*). It will be seen from Fig. 4a, that the grain layer is well merged into the corium, and Fig. 4b shows the same leather when bent;

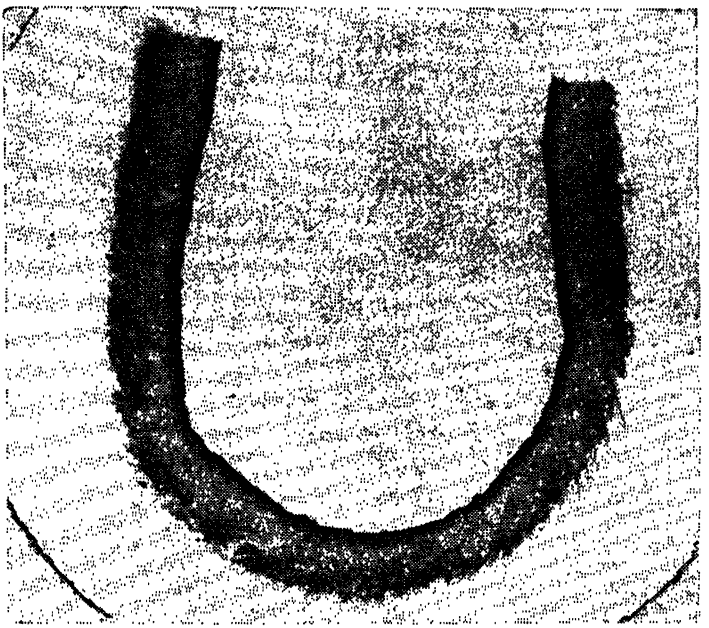

Fig. 4b. *The same leather as in Fig.* 4*a.* (*after finishing*) *in bent condition* (×6.5)

because of the firm attachment of the grain fibres to the corium practically no wrinkles have developed. When the attachment of the grain to the corium is less firm, it is easier for the grain fibres to change their relative position than to be compressed (Fig. 5). This condition of fibres will produce a coarse break. It will be observed from Fig. 5, that the merging of the grain into the corium is very poor *i.e.*, the attachment between the grain and corium is not firm. When this leather is folded with grain inward, the grain layer has no other alternative but to form wrinkles due to compression as in Fig. 3a. So it can be said that the natural grain tightness (the natural tension of the grain layer) and the merging of the grain into the corium (the attachment of the grain to the corium) play the guiding role in the production of tight-grained leat. th fine break.

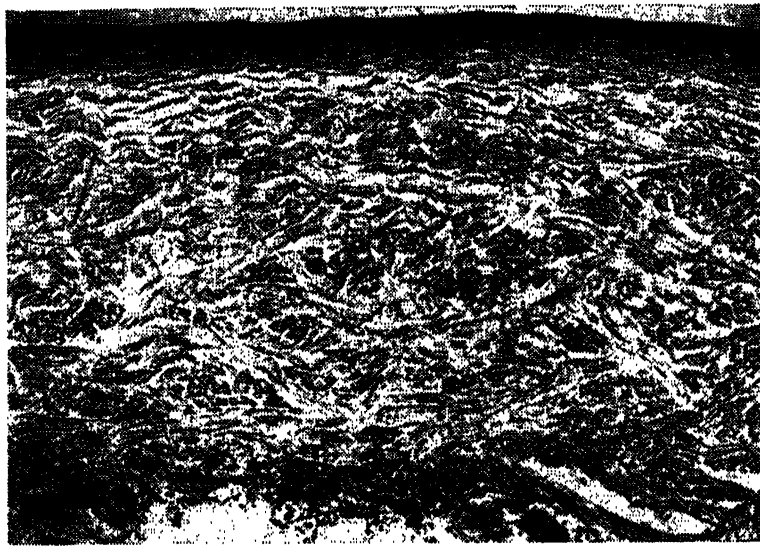

Fig. 5. *Section of leather showing poor merging of the grain into the corium* (× 36)

It was found difficult to assess the compactness of the fibre texture in raw hide from its cross sections. So an indirect method was followed involving water absorption by Kubelka apparatus. It was observed that water-uptake varied not only from hide to hide but also from different locations of the same hide, and the looser area absorbed more water than the compact area. It was also observed by Mitton[4] that water absorption depended upon location in the hide, and higher water absorption indicated loose and open structure. So water absorption method was adopted as a measure to assess the compactness of the fibre structure of raw hide.

It was observed that although the moisture content of wet salted hide varied from 43 to 47%, the average moisture content in the butt was about 45% and that in the belly was about 46%. Nandy[5] *et al* also observed the same condition It was further observed that in most of the cases water absorption reached about the maximum level after soaking the hide in water for 2½ hr. If the hide pieces were allowed to keep in water for more than 2½ hr, water absorption showed a very small increase which was negligible. So in this work water-uptake after soaking for 2½ hr was taken as a measure for compactness of fibre structure of raw hide. It may be questioned here that the water-uptake of different hides determined by this method cannot give a comparative result because the initial moisture content of the hides varies between 43% and 47%. Water-uptake, if determined on moisture free basis,

could have given more accurate result. This is conceded but it will require 48 to 72 hr whereas the present method takes only 2½ hr. The object of this work was to find out a method by which a tanner could assess the quality of raw hide in a very short period. Keeping this in view the present method was developed.

It will be seen from Table 2, that the hides which have produced full and soft leather with good temper in the butt, are Nos. 2, 3, 5 and 6. A reference to Table 1 will show that water-uptake (in 2½ hr) in these hides at the butt varies between 31% and 40% which is quite low comapred to water-uptake in other hides for the same period. And with regard to fullness and feel, these hides have also produced superior leather. This indicates that packing of fibre bundles in those hides is very compact. The leathers which are fairly full with a soft and mellow feel but without temper in the butt are Nos. 1, 4, 7 to 10 in group 'A', Nos. 11, 12, 14, 15, 18 and 19 in group 'B', and 26 in group 'C'. It will be evident from Table 1 that water-uptake in most of these hides, in the butt, varies approximately from 41 to 50% which is quite higher than the water-uptake in hide Nos. 2, 3, 5 and 6 ; the quality of leather made from these hides (Table 2) as regards fullness and feel is also inferior to those leathers made from hide Nos. 2, 3, 5 and 6. This shows that the fibre texture of these hides is less compact than those which have produced full and soft leather with good temper. Again, the leathers which are slightly empty with very soft feel in the butt, are Nos. 13, 16, 17 and 20 in group 'B', and 21, 22 and 28 in group 'C'. Water absorption in these hides in the butt is quite high varying between 51% and 60%. And the hides which have produced empty leathers with raggy feel in the butt are Nos. 23, 24, 25, 26, 29, and 30 in group 'C'. Water absorption in these hides as will be evident from Table 1 is very high and varies between 61% and 70% approximately. The fulness and feel of these leathers as per Table 2 are the poorest. From the above discussion, it will appear that as the water absorption in the hide increases, the fulness and feel of the leather decreases *i.e.*, the looser the fibre structure softer is the feel and thinner the substance of the leather. A comparison of the data in Tables 1 and 2 will show that similar conditions also exist in the belly region. It can thus be concluded that the grain quality and feel of upper leather are mainly dependent on three inherent characteristics of raw hides : (i) natural tightness of the grain (ii) merging of the grain into corium and (iii) compactness of the fibre structure as indicated by water-uptake. Although any one of the above factors may intensify or minimise the effect of other factors, the quality of leather is influenced by the cumulative effect of all the factors.

As the grain quality and feel of leather are primarily dependent on the cumulative effect of the above three factors, an attempt has been made to find

out a method for numerical assessment of raw hides by allotting arbitrary values to each of the factors in the same way as Marriott[6] suggested for the assessment of sole leather. The assessment Table is given below :

Factors	Assessment Number (Values)				
	8	6	4	2	0
Natural tightness of the grain (%)	31 and above	25-21	15-11	5-1	0 and <0
Apparent merging of the grain into the corium	Very Good	Good	Fair	Poor	Very poor
Water-uptake of W/S hide by Kubelka apparatus in 2½ hr (%)	35 and Less	41-45	51-55	61-65	71 and above

It will be seen from the above Table that assessment values are grouped into 5 columns, the maximum value being 8 and minimum 0, with a difference of 2 marks between any two adjacent columns. The maximum natural grain tightness found in cattle hide during this study was 31% (hide No. 2). So this high figure for natural grain tightness (31% and above) is placed in the first column bearing 8 marks. Although the minimum natural grain tightness found in the experimental hides was 7%, this figure is not placed in the last column, because the natural grain tightness in very old animals may be even 0%. This is possible because when the animal becomes very old, the grain layer loses its elasticity and the area of the split grain and the corium becomes more or less the same. Montagna[7] also observed that in old age, with the loss of elasticity and decrease in the fat content, the skin sags and becomes furrowed by wrinkles. So 0% natural grain tightness is placed in the last column bearing 0 mark. Natural grain tightness from 1 to 30% is divided equally among the intermediate columns having 5 ranges in each column. The grain-merging in cattle hides normally varies from very good to very poor. So, the terms very good, good, fair, poor and very poor are selectec..

Now the compactness of the fibre structure expressed as percent water uptake in 2½ hr varies between 30% and 81% in wet-salted cattle hides as will be evident from Table 2, provided the hides are not dried up even partially. Though the minimum figure for water-uptake obtained in cattle hides is 31% (hide No. 3), the figures below 35% are very rare. So the figures 35% and less are placed in the first column bearing 8 marks. Again, the highest figure for water-uptake found in the cattle hides is 81% and that also is obtained

in one hide only. Other higher figures are above 70% but mostly below 80%. So 71% and above are placed in the last column bearing 0 mark. The figures from 36 to 70% are divided equally between the intermediate columns having 5 ranges in each column. Now the average value of the three columns for three factors viz., natural grain tightness, merging of the grain into the corium and water-uptake will be the assessment value which would give an indication of hide-quality. The average assessment value for varying hide-quality is given below :

Assessment value	Hide quality
0 to 3	Poor
3.1 to 5	Fair
5.1 to 7	Good
7.1 to 8	Very good

In the light of this study it is not claimed that the method suggested for assessment is fool-proof. No physical assessment for hides is cent per cent correct for obvious reasons. If this method gives correct indication even upto 80%, it will help the tanner to a great extent.

Acknowledgement

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Curing Raw Hides Microbiological Defects, their Diagnosis and Prevention

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The study on the microbial colonies in raw hides revealed that raw hide should be cured immediately on the day of taking off if not stored at lower temperature. At 10°C and — 1°C it was possible to store the hide for one week and 3-4 weeks respectively without worsening of the quality but at — 1°C the hide fibres split due to the growth of ice crystals.

To prevent the formation of red colour or the overheating of raw hide, an addition of 2% soda and 2% naphthalene or 2% Zn—diethyldithiocarbamate on salt weight was found to be satisfactory. With a view to evaluate the quality of cure and the degree of bacterial attack on hide, estimations were made on the quantitative determination of bacteria, solid content, nitrogen and tryptophan present in aqueous hide extract and the *p*H of this extract and tryptophan content was found to be the proper index of the deterioration of raw hide. Determination of tryptophan is recommended as a simple and objective method to evaluate the quality of cured hide.

Hide proteins are the suitable substrates which are easily hydrolytically split by a number of enzymes. The effect of microbial activity esults in proteolysis leading to degradation of the hide substance. The role of halophilic and non-halophilic bacteria, producing or not producing colored spots on a alted hide, is not quite clearly explained, because the different species of bacteria can manifest themselves concurrently so that their hydrolytic effect is overcovered.

During the last decade many papers were dedicated especially to halophilic microorganisms and to the problem of the red colouration of salted hides, as forexample papers of Boidin and Ginisty[1], Formisano, Simoncini and Meduri[2,3,4]. Bartels and Hadlok[5], Vivian[6] and Gastellu, Julien and Prevot[7]. The importance of the problem was further stressed by Nandy *et al* [8,9].

In the present work the problems of microbial attack of the raw hide cured by salt with and without bactericides have been studied. At the same time an attempt has been made to diagnose the occurrence of hydrolytic processes.

Materials and Methods

For analyses, fresh cattle hides and pig skins immediately after take off in the slaughter house were used. The hides and skins were cured with (i) salt (ii) salt in admixture with 2% soda and 2% naphthalene and salt in admixture with 2% Zn-diethyl dithiocarbamate. They were stored for nine months at 22°C. The following microbiological analyses were performed :

Determination of the number of bacteria by cultivation on meat peptone agar (MPA) for 48 hr at 57°C and for 72 hr at 22°C [10].

Determination of the number of halophilic bacteria by cultivation on MPA with 20% salt for 7 days at 37°C.

Determination of the number of proteolytic bacteria by cultivation on meat peptone-gelatine for 4-7 days at 15°C [10].

The determination of the total number of bacteria by counting them in a Burker chamber.

The effect of various curing agents like soda, naphthalene, Zn-diethyldithiocarbamate and boric acid on the growth of *Bacillus megatherium* was also studied using growth-curves[12] to confirm the effect of these agents on microbes. All microbiological analyses were performed on a water eluate, prepared by shaking 20 g raw hide in 200 ml distilled water for 3 hr. The following analyses were made : determination of solids, nitrogen (by Kjeldahl method), changes of *p*H value in the eluate, tryptophan reaction by the method of Voisinet[11]. The tryptophan reaction method was as

follows: An aliquot of the eluate was concentrated on a water-bath to complete dryness. The dry residue was dissolved in 1 ml 30% NaOH solution. One drop of 1.25% formaldehyde solution and 10 ml concentrated HCl were added. After 10 min, 5-7 drops of 0.05% $NaNO_2$ solution was added. A blue-violet ring indicated a positive reaction.

Results and Discussion

It is evident from the inplant experiences up to this time and from laboratory tests, that it is necessary to cure the hide after take off the same day, because after 20 hr the uncured hides give off a putrid odor. We have followed the storing of uncured hides at lower temperatures. At 10°C it is possible to preserve the hides without deterioration in the quality for a maximum period of 1 week. Thereafter the hides begin to putrefy. Hides stored at—1°C remain without infection for approximately one month. Later, they too start to give a putrid odor due to development of proteolytic microflora. Furthermore, the outside parts of the hides are damaged by ice crystals, found in the tissue. For these hides, we have performed the analyses, given in Table 1. These analyses show the number of microbes, the solids present in eluates and the reaction of tryptophan. All these characterise the degree of the microbial degradation of hide substance. This table shows also that, if the number of microbes grown on MPA at 37°C during 48 hr goes beyond 1.0×10^8 bacteria per 1 g of hide, we have to do with a hide considerably infected having a putrid odor. In any case, it is not possible to store such a hide longer. This biological degradation is confirmed also by a qualitative tryptophan reaction. While in fresh hides or in hides stored at about 0°C this reaction is negative, in hides with an advanced bacterial infection, it is positive. The smaller the quantity of eluates needed for a positive reaction, the more intensive is the microbiological degradation of hides. The differences, found by determination of solids in the eluate of tested samples, are less expressive. The augmentation of solids is evident only after a longer time and during the action of microbes on a hide at higher temperatures. The augmentation is not evident even after 18 days of storing at 1°C, even though it is true that the number of bacteria is increasing. This is obviously due to the fact, that the micro-organisms are developing but their degradative and hydrolytic effect is rather slackened by the low temperature. In contrast to this, values found by the determination of tryptophan, characterise the microbiological attack even at a level for which other methods do not still give some positive reaction. Further, we have ascertained some physiological groups of microbes in hides, cured by the current inplant method using sodium chloride. The values are given in Table 2.

TABLE 1

The role of the time of storage on the microbial degradation of uncured calf skin

Incubation period	Temperature of storage (°C)								
	22			10			—1		
days	Number of bacteria per 1 g	Solids of eluate in 1% of dry matter	Tryptophan reaction	Number of bacteria per 1 g	Solids of eluate in 1% of dry matter	Tryptophan reaction	Number of bacteria per 1 g	Solids of eluate in 1% of dry matter	Tryptophan reaction
1	1.9×10^8	2.32	+	3.1×10^7	2.36	—	2.7×10^7	2.36	—
12				1.6×10^8	5.60	+	6.2×10^6	2.44	—
18							1.8×10^8	2.24	—

+ Positive

— Negative

TABLE 2

Microbial colonisation of hide cured with sodium chloride

	Cattle hide	Calf skin	Pork skin
Number of bacteria per 1 g MPA, 37 C, 48 hr eluate	$2.5 \times 10^7 - 5.0 \times 10^8$	$2.0 \times 10^7 - 2.8 \times 10^8$	$1.0 \times 10^5 - 5.9 \times 10^7$
Number of bacteria on 1 cm^2 of flesh side, MPA, 37°C, 48 hr tampoon method	$2.2 \times 10^3 - 4.3 \times 10^4$	$1.8 \times 10^2 - 5.0 \times 10^2$	$1.0 \times 10^2 - 2.1 \times 10^3$
Halophilic bacteria per 1 g MPA with 20% of NaCl 37°C, 7 days	$1.0 \times 10^5 - 5.0 \times 10^7$	$1.0 \times 10^5 - 3.1 \times 10^6$	$1.0 \times 10^5 - 2.0 \times 10^6$
Proteolytic bacteria per 1 g of MPA 15°C, 7 days	$4.0 \times 10^4 - 6.8 \times 10^5$	$5.2 \times 10^4 - 8.6 \times 10^5$	$3.0 \times 10^4 - 1.0 \times 10^5$
Psychrophilic bacteria per 1 g of MPA, 22°C, 72 hr	$1.0 \times 10^8 - 8.0 \times 10^8$	$7.7 \times 10^7 - 5.8 \times 10^8$	$1.2 \times 10^8 - 6.3 \times 10^8$
Number of bacteria per 1 g (method of Burker chamber)	$1.4 \times 10^9 - 1.0 \times 10^{10}$	—	—

It is evident from this table, that one obtains the maximum number of microbes by counting them in the Burker chamber, wherein both the living and dead cells are counted on nutritional media, we can ascertain only certain types of microorganisms. The table further shows that in the inplant curing method, the number of microbes on the hides reach rather high values and that there exists no difference between the sorts of hides (pig and calf skins and cattle hides have the same value when used as a substrate).

During the determination of microbes by inoculation on MPA, different values are obtained with the microbes present in an eluate of a given amount of hide. By the wip-tampoon method the microbes of the uppermost part of the flesh side and that too only approximately in consequence of the unevenness of the flesh side, are estimated. Most of the bacteria present in hide and capable of growing at 22°C are classified as psychrophilic ones. Included in this group are also proteolytically active strains. In the microflora, mentioned it is necessary to include also the group of halophilic bacteria, which form, however, only a part (less than 10%) of microbial population found on a hide, cured well under inplant conditions. Under suitable conditions, this companionship may help a great way to the formation of red pigmentation on flesh side of the hide. We have already ascertained that at the beginning of this process the halophilic microbes make 50% of the inhabitation, found by cultivation on MPA both without sodium chloride and with 20% of sodium chloride.

Similar analyses of microbiological attack and inhabitation on hides, cured with sodium chloride alone and with sodium chloride containing antiseptics like soda plus naphthalene and Zn-diethyldithiocarbamate, are recorded in Table 3.

TABLE 3

The effect of different curing methods on the microbial corrosion of pork skins stored for 9 months at 22°C

	Method of curing		
	NaCl	NaCl plus 2% Zn-diethyl-dithiocarbamate	NaCl plus 2% naphthalene plus 2% soda
Number of bacteria per 1g MPA, 37°C, 48 hr	9.7×10^7	3.6×10^7	1.8×10^7
Solids of eluate, in % of dry origin material	9.2—12.5	5.9—7.0	10.3
Reaction on tryptophan	+	—	—
Visual estimation	Quite red	No spots	Red bordering

The values in Table 3 characterise the microbial colonisation, the solids of eluate and the reaction on tryptophan in samples after a storage of 9 months. These tests show quite precisely, that the most microbially attacked hide is the one cured with sodium chloride alone and therefore also the most degraded. The hides cured with an addition of bactericidal matters to the curing salt show a lower colonisation and are less biologically affected. This can also be seen visually.

Samples of hides, cured by the above methods were stored for 14 months. After this time, it was found that, the inplant cured hides became quite red and slimy and started losing hair and succumb to proteolysis ; the hides cured by salt with the addition of naphthalene and soda succumb to microbial corrosion only in the form of bordered spots and the hides cured by salt with the addition of Zn-phenylethyl- or diethyldithiocarbamate remained practically in good condition without evidence of proteolytic processes. The outstanding effect of Zn-salts of the dithiocarbamic acid and of naphtalene with soda against the red pigmentation of salted hides is given by their bactericidal effect. It is known that Na-diethyldithiocarbamate is an enzymatic poison[13,14], acting in a way to form an undissociable complex with the copper in the prosthetic group of enzymes. By this, it is possible to explain also the effect of Zn-diethyldithiocarbamate against microorganisms on salted hides.

On the basis of these determinations, we performed some inplant curing tests on cattle hides and calf skins. We ascertained, that after 6 months' storage during the summer (May to October) 50% of hides cured only with salt were quite red with loosening of hair, while hides cured with the addition of soda and naphthalene and Zn-diethyldithiocarbamate to the salt, only 3% of the hides were red with partial loosening of hair. It is evident, therefore, that addition of antimicrobial agent to sodium chloride can considerably improve the curing efficiency of salt.

To make the effect and influence of added matters to a curing salt more clear, we made some determinations on the influence of solutions and suspensions of antimicrobial compounds against *B. megatherium*. For this determination the method of growing curves[12] was used. The results, given in Table 4, show clearly that soda with naphthalene influences the microorganisms immediately by a toxic shock and at a concentration of 0.25% each makes the medium quite sterile after 24 hr. The initial effect of Zn-diethyldithiocarbamate is, however, more slow than in the case of naphthalene and soda. Boric acid and naphthalene although recommended[6] to be effective in preventing 'red-heat' and 'red spot' are found to possess rather weak inhibitory action on *B. megatherium*. From the accomplished analyses it is

evident, that the addition of the above mentioned compounds to the salt is quite necessary in view of their bactericidal properties which hinder the red colouring and overheating (loosening of hair) in hides.

TABLE 4

The effect of bactericides on the growth of *B. megatherium*

Concentration	Number of bacteria per 1 ml	
	0 hr	24 hr
Soda plus naphthalene		
1.0 each	0	0
0.5 „	10	0
0.25 „	2.6×10^3	0
0.05 „	1.4×10^3	2.1×10^5
Control	1.4×10^5	2.3×10^5
Zn-diethyldithiocarbamate		
2.0	3.3×10^3	0
1.0	6.8×10^4	0
1.5	8.2×10^4	0
0.1	1.0×10^5	0
Control	1.2×10^5	2.5×10^5
Boric acid plus naphthalene		
1.0 each	4.7×10^4	1.0×10^3
0.5 „	6.7×10^4	1.3×10^3
0.25 „	7.0×10^4	4.5×10^4
0.05 „	8.3×10^4	6.1×10^4
Control	1.0×10^5	1.4×10^5

Besides the improvement of curing, our aim was to present to the industry an effective and simple method for quality control in the curing operation. The determination of the amino acid tryptophan by means of the Voisenat reaction appears to be the most convenient index. We have determined also the solids of an water eluate, the content of nitrogen in the eluate by the Kjeldahl method and the changes in the *p*H value of the e uate. The determinations of solids, nitrogen and the *p*H values are not a suitable index for the cured hides, because these values are influenced not only by the sort of curing and by the degree of biological degradation, but also by the heerogeneity in the composition of hides.

On the basis of the obtained results, we can make a conclusion, that one can characterise the microbiological attack on a hide by means of the reaction to tryptophan as follows :

1 A well cured hide, eventually a fresh one (with a bactericidal and bacteriostatic curing)	—the reaction is negative even at a concentration of 50 ml of eluate
2 A hide without a clear damage, but characterizied by development of microbes (the curing is not even a bacteristatic one)	—the reaction is positive after a concentration of 30 ml (and more) of eluate
3 A hide with numerous spots on the flesh side, sometimes even with loosened hair (the curing is not sufficient)	—the reaction is positive after a concentration of 10 ml of eluate (and more)
4 A hide clearly putrified	—the reaction is positive either in 1-3 ml of eluate without concentration or in 4-9 ml of eluate after concentration.

The positive reaction for tryptophan, found after concentrating the eluate to 10 ml is characteristic of hide clearly damaged by hydrolytical action of microbes. In the case of a positive reaction in samples of 11 to 30 ml of concentrated eluate (under mentioned conditions), it is necessary to admit that the hide is microbiologically affected and that the occasional defects could take their origin in consequence of this attack. It is necessary to remark, that such an estimation of an microbiological attack fits only with regard to a hide, but not to the produced leather. In the grain layer of a leather, there are mainly residues of hairs, hydrolysed by sodium sulphide. These residues contain tryptophan and therefore, it is possible to find in the grain layer of leathers a positive reaction to tryptophan.

In the case of a hide, the positive reaction for tryptophan is, on the contrary, caused by the presence of microorganisms and their proteolytic action, eventually by the hydrolytic cleavage of the hair follicles and roots.

The presented method of a qualitative determination of tryptophan is remarkably simple and very reliable. We are recommending it therefore as an objective method for controlling the quality of cure and for controlling the degree of microbiological attack on a hide.

Acknowledgements

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Effect of Delay in Cure on the Rate of Vegetable Tanning and on the Properties of Sole Leather Made from Buffalo Hide

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Buffalo hide, staled for 24 hr at about 30°C did not appreciably deteriorate in quality but when staled for 48 hr or more it was degraded to a considerable extent. The rate of tanning was found to be comparatively rapid in the case of staled hides than in fresh hide.

Leather yield was not affected upto 24 hr delay in cure but was reduced when staled for 48 hr or more. Certain physical properties of the sole leather e.g. apparent density and abrasion resistance were found to be practically unaffected by staling whereas water absorption was increased after 48 hr with the increase in delay in cure. Chemical composition of the leather was influenced only slightly but the general appearance and gradation of the leathers were considerably affected when staled for 48 hr or more.

Delay in cure may affect the quality of hide which in turn may influence the rate of vegetable tanning. Two large scale experiments were conducted by British Leather Manufacturers' Research Association[1] in conjunction with Penketh and Camden Tanning Companies with hides staled for 3 to 14 days. Leathers produced from staled hides were found damaged on the grain with little change in physical properties. Stather and Slyter[2] reported that white weight gain and leather yield did not vary between fresh and 24 hr staled hide cured either by salting or brining. In a later study Stather and Herfeld[3] extended the staling period to 72 hr and observed no variation in leather yield,

specific gravity, tensile strength, stretch and chemical composition between fresh and staled hides. Leathers produced from 72 hr staled hides, however, showed a pitted grain and had a comparatively greater capacity for water absorption than other samples. It is well recognised that the intensity of the effect of delay in cure depends to a great extent on the temperature[4] existing during staling and in a tropical country like India, staling may have a pronounced effect on leather quality. Moreover, a good proportion (80%) of the Indian buffalo hides are obtained from fallen animals which are often staled for a period of about 24 hr and sometimes even more. The effect of delay in cure on the rate of tanning, leather yield and on the properties of sole leather was studied and the results are reported in this paper.

Materials and Methods

The quality of hide was determined by estimating the extractable hydroxyproline nitrogen present in soak water[5].

Different tanning procedures were followed by different investigators to study the rate of tanning. On many occasions the concentrations of the tan liquors were maintained at the same level throughout the tanning operation. But in practice, a weak liquor is used for early tannage and the strength of the liquor is gradually increased till the tanning is completed. Moreover, the composition of the tan liquor may appreciably change if the same liquor is used for tanning, either alone or after being strengthened by addition of fresh tannin. Considering the above points the following procedure was adopted for tanning in the present study.

Salted buffalo hide samples were washed well and then soaked overnight in water. They were then limed in a liquor containing sodium sulphide — 0.25%, lime — 6%, old lime liquor — 150% and water — 150% (all on soaked weight) with proper handling. After 4 days, they were unhaired and then relimed for 3 days with lime — 10% and water — 300%. Then they were fleshed, washed, delimed completely with 1% ammonium sulphate and then put in a 10° Bk liquor made up of mimosa spray dried extract. Pelts were handled twice daily. Next morning the liquor was adjusted to the initial Bk. strength by adding a fresh quantity of extract powder. After two days of handling in 10° Bk liquor the pelts were transferred to a 20° Bk liquor freshly prepared with extract powder. The pelts were handled as before and the strength of the liquor was readjusted on the next morning. After 2 days the pelts were transferred to a new liquor of 30° Bk. In a similar way, pelts were treated for two days each with liquor of 40, 60, 80 and 100° Bk. Tanning was

done at room temperature (30° ± 2°C) at the normal *p*H of the tan liquors. Tanned pelts were treated for two more days in a liquor of 100 ° Bk and then washed well, bleached, loaded, oiled, set well and then dried. The leathers were finished as usual after rolling.

Mezey[6] estimated the rate of diffusion of tannin by staining histological sections at regular intervals and measuring the depth of penetration under the microscope. This method gives an indication of the extent of tanning during early stages *i.e.*, till the pelt is struck through. The increase in T_s during tannage might be attributed to crosslinking formation during early stages of tanning and this was considered[7] to give a measure of tannage. Holmes and Woollenberg[8] recommended a method for the estimation of rate of tanning by measuring the weight gained by the pelt in the liquor during tanning till the equilibrium was reached. It was also considered that the amount of tannin fixed by the pelt at any time in the course of tanning will give further evidence of the extent of tanning.

A preliminary investigation revealed that an estimation of T_s and fixed tan at regular intervals during tanning provided adequate information about the rate of tanning under the present experimental conditions. The extent of tannin penetration as determined histologically was found to be related to the rise in T_s and the maximum T_s was reached when the pelt was just struck through. Because of appreciable difference in tanning procedure Wollenberg's method was considered unsuitable for the present study.

The fixed tan was estimated in an indirect way. Samples were cut at regular intervals and were dried in the laboratory (about 30°C) and then made into small pieces (5 mm sq). They were extracted with disilled water (leather : water ratio was 1 : 5 w/v) for 2 hr in a mechanical shaker to remove the water solubles. The leather pieces were dried at room temperature and then at 105°C for 6 hr. Hide substance was then determined by Kjeldahl method. The leather pieces being free from moisture and water solubles and considering the fat and ash contents to be roughly constant, fixed tan may be determined as

$$100 - \text{Hide substance} = \text{Fixed tan}$$

In order to find out the rate of increase in fixed tan on comparative basis, values for fixed tan obtained by the above method were considered acceptable.

Water absorption was determined by the Kubelka method ; apparent density was determined by mercury displacement method and abrasion was measured by an abrasion tester after 400 revolutions.

Moisture, fat, water solubles, hide substance and degree of tannage were determined according to the standard methods.

Results and Discussion

A freshly slaughtered buffalo hide was washed, green fleshed and then cut into experimental pieces as shown in Fig. 1. Hide samples were made

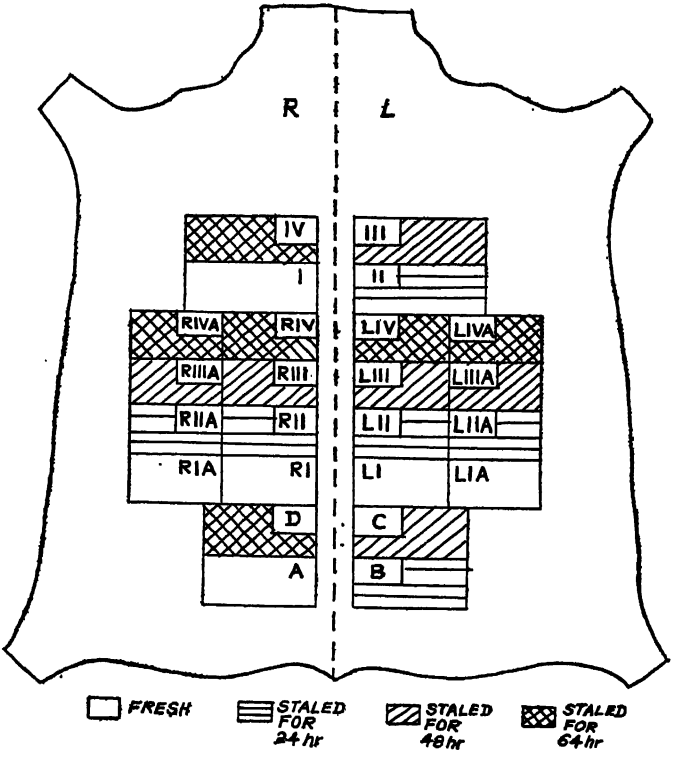

Fig. 1. *Diagram of the hide showing sampling positions*

into a pile flesh side up and kept inside a moist chamber at room temperature (29 ± 1°C) for staling. Samples were taken after staling for 24, 48 and 64 hr, sprayed with an antiseptic solution (Topane WS) to prevent further bacterial action and then cured with common salt. They were stored for 7 weeks and then taken for quality assessment and tanning. Samples I, II, III and IV, staled for different periods, were taken for the quantitative determination of hide quality. Data on the hide quality are given in Table 1.

TABLE 1

Quantitative assessment of hide quality as affected by delay in cure

Period of staling (hr)	Hair-slip	Extractable hydroxyproline (% of total N)
Fresh	No	0.002
24	No	0.002
48	Yes	0.021
64	Yes	0.038

Extractable hydroxyproline nitrogen present in hide samples shows that fresh and 24 hr staled hides stored for a period of 7 weeks after salting are capable of producing good leathers but the hide samples staled for 48 and 64 hrs deteriorate in quality to a considerable extent resulting in only poor quality leathers.

Samples A, B, C and D were taken to study the effect of staling on the rate of tanning. Samples RI, RIA ; RII, RIIA ; RIII, RIIIA ; and RIV, RIVA; staled for varying periods, were considered as experimental samples and the corresponding fresh samples from the left side served as controls. These samples were used to study the leather yield and certain physical and chemical properties of the leathers.

Hide samples were tanned according to the procedure mentioned earlier. The pelt-tan liquor ratio used was 1 : 8. The extent of tannage was measured from T_s and fixed tan determinations at regular intervals and the data obtained are given in Tables 2 and 3.

TABLE 2

Effect of staling of buffalo hide on the rate of vegetable tanning as determined by shrinkage temperature

Strength of tan liquor (°Bk)	Period of tanning (days)	Increase in T (°C)			
		Fresh	Staled		
			24 hr	48 hr	64 hr
Delimed	—	0	0	0	0
10	2	6.0	7.0	6.5	6.0
20	4	15.0	18.5	21.5	23.5
30	6	22.0	24.0	24.5	24.5
40	8	23.5	23.0	23.5	24.0
60	10	21.0	21.0	21.0	22.5
80	12	19.5	20.0	19.5	20.0
100	14	18.0	18.0	18.5	19.0

TABLE 3

Effect of staling of buffalo hide on the rate of vegetable tanning as determined from tannin fixation (% fixed tan on dry weight)

Strength of tan liquor (°Bk)	Fresh	Percent fixed tan on dry weight of leather		
		24 hr	48 hr	64 hr
Delimed	0	0	0	0
10	30.03	33.76	35.62	37.15
20	40.41	41.89	43.41	43.45
30	47.39	48.52	49.29	48.62
40	48.44	50.18	48.48	48.10
60	48.15	48.45	48.80	48.50
80	48.72	49.89	49.02	49.07

It is apparent from Table 2 that the rate of tanning is comparatively slow in case of fresh hide where the maximum T_s has attained after 8 days. In case of staled hide samples T_s has reached its maximum after 6 days although plotting of the data may probably show that the rate of tanning slightly increases with the increase in staling period. Data on tannin fixation reveal that the rate of tanning, independent of staling period, is quite rapid during the early stages of tanning (upto 6 days) after which the rate of tannin fixation is rather slow. Fixation of tannin is found to be comparatively rapid in case of highly staled hide samples during early tannage but this variation in tannin fixation becomes less apparent at the later stages of tanning. This increased rate of tanning in heavily staled hides is probably due to more loose and opened up hide structure caused by microbial degradation of hide which permitted easy diffusion of tannin into the pelt. Slightly higher diffusion and fixation of tannin in highly staled buffalo hide may conveniently be correlated with more swelling[9] and salt absorption[10].

The weight yields of leather calculated as per cent on green, staled and pelt weight are presented in Table 4 . Data given in the table are the average values of two samples in each case.

TABLE 4

Effect of staling of buffalo hide on the weight yield of vegetable tanned leather*

Period of staling (hr)		Weight yield of leather (%)		
		Calculated on green wt	Calculated on staled wt	Calculated on pelt wt
Fresh	Control	80.07	80.07	66.37
	Experimental	79.68	79.68	66.90
24	Control	78.04	78.04	66.22
	Experimental	78.32	79.29	66.56
48	Control	76.25	76.25	65.04
	Experimental	68.67	72.16	62.87
64	Control	76.59	76.59	66.36
	Experimental	65.63	69.19	81.42

* Average yield of two samples.

Weight yield, calculated either on green, staled or pelt weight, remains unaffected upto 24 hr staling, but when hides are staled for 48 and 64 hr, weight yield decreases with staling period. Loss in weight yield appears to be compensated to some extent when calculated on staled weight and is further compensated when calculated on limed wieght (usual commercial practice). For example, a hide staled for 48 hr shows 7.58% reduction in leather yield than fresh (control) hide when expressed on the green weight but the same hide appears to produce only about 2% reduction in yield when expressed on pelt weight.

Certain physical and chemical properties of the leathers obtained from staled and fresh (control) hide samples are tabulated in Tables 5 and 6.

TABLE 5

Effect of staling of buffalo hide on some physical properties of finished sole leather.*

Physical properties		Fresh	Period of Staling (hr)		
			24	48	64
Abrasion (Inch/400R)	Control	0.093	0.102	0.102	0.095
	Experimental	0.084	0.093	0.092	0.062
Water absorption (%)	Control ½ hr	31.4	30.2	32.9	32.8
	24 hr	45.8	46.1	45.1	46.1
	Experimental ½ hr	31.2	29.7	38.4	42.9
	24 hr	46.2	45.9	50.2	52.6
Apparent density	Control	0.987	0.987	0.990	0.998
	Experimental	1.018	1.000	0.997	0.975
Apparent density × hide substance	Control	40.27	40.17	39.60	40.12
	Experimental	41.23	40.10	38.10	37.54

*Average values of two samples.

TABLE 6

Effect of staling of buffalo hide on the chemical composition of sole leather

Chemical analysis		Fresh	Period of staling (hr)		
			24	48	64
Moisture (%)	Control	12.9	12.7	12.7	13.2
	Experimental	12.3	12.7	12.5	12.6
Oils and fats (%)	Control	2.2	2.1	2.2	2.6
	Experimental	2.2	2.2	3.4	2.2
Water solubles (%)	Control	17.8	17.8	17.4	17.0
	Experimental	18.0	17.7	18.0	18.4
Hide substance (%)	Control	40.8	40.7	40.0	40.2
	Experimental	40.5	40.1	39.0	38.5
Degree of tannage	Control	64.5	65.6	69.3	67.1
	Experimental	66.7	67.6	72.1	73.5

Water absorption is found to remain unaffected upto 24 hr staling but with further increase in staling water absorption appears to increase first slowly and then appreciably. As water absorption depends on the fibre structure of the leather it may be assumed that a leather with loose and empty structure produced from highly staled hides will absorb more water.

As regards apparent density, no significant difference can be noted between the experimental and control leathers. It is expected that an empty fibre structure will show less apparent density but during vegetable tanning a pelt with loose fibre structure will absorb more water solubles. The density of the water solubles being roughly the same as that of fibres, the possible decrease in apparent density in leathers from highly staled hides seems to be mostly compensated. Bradly[11] pointed out that the product of percentage hide substance and apparent density might give better indication about the structure of the leather. A comparison of such values (hide substance × apparent desnity) between the control and experimental leathers shows that leather structure is considerably affected by 48 and 64 hr delay in cure.

It is striking to note that abrasion of the leathers is not influenced upto 48 hr staling but seems to be less in leathers from 64 hr staled hides. While comparing the properties of sole leathers made from staled and fresh hide Robertson[1] observed that bend samples from most of the staled sides possessed surprisingly good resistance to abrasion. Staled belly samples, however,

showed a lower abrasion resistance. This high abrasion resistance was explained by her due to bacterial degradation during staling which resulted in glueing together of the corium fibres during manufacturing process.

While comparing the chemical composition of the control and experimental leathers it may be noted that water solubles slightly increase and hide substance slightly decreases with the increase in staling period. Degree of tannage appears to be slightly higher in case of highly staled hide.

General appearance and assessment of the leathers are found to be normal upto 24 hr staling but are affected due to considerable grain damage and are graded only as rejections when staled for 48 hr or more. Flexibility is also found to be influenced by considerable staling and leathers from 48 and 64 hr staled hides appear to be somewhat soft.

It is thus apparent from the present investigation that sole leather making potentiality of buffalo hide is practically unaffected upto 24 hr staling but is deteriorated progressively with the increase in staling period although the physical and chemical properties of the leathers are not appreciably affected upto 48 hr staling.

Acknowledgement

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DISCUSSION

SESSION II

Y. Nayudamma (*Central Leather Research Institute, Madras*): Mr. Schroeder, is there any improved method of flaying hides and skins other than the conventional methods ? Is it possible to flay in a different manner so that a hide is brought in a better form without having loose bellies ?

L. Schroeder (*Hude-Centralen, Glostrup, Denmark*) : I have been in a tannery for many years and I know exactly what the tanners want. So, in collaboration with the Swedish people, we modified the trim pattern by removing the head (leaving cheeks on the hide), hooves and the legs beyond the knee. This we call ' Scandinavian trim ' and has been introduced in Denmark, Sweden, Finland and also in Norway. The quality of the hide is not influenced whether the carcass is flayed in hung up or laid down position. Of course, the improvement in the quality of the hides has been attempted by using flaying machines. Flaying machines of different makes, *e.g.*, West German, English, French and Italian are available but in Denmark we use West German make E.F.A. machines. Recently we got a new machine from Switzerland.

The new thing in this field is the development of a machine in Sweden by which the whole hide is cooled down and flayed mechanically having no fat and flesh on it. It takes shorter time for flaying ; the hides have no cuts at all. The third machine produced by them has been bought by us and I think within few years this will spread out all over the world. They have worked on it for years and only recently, (1½-2 months), this machine has been put in the market.

J. K. Khanna (*Central Leather Research Institute, Madras*) : Bellies and shanks on Indian Cow hides are very loose and thin and when cut into crops or croupons we get narrow bellies (about 6 inches). Could we change the flaying pattern in such a way that we get two bellies in a single piece and the crop as such ? Has any work been done in this direction ?

G. Toth (*Chemolimpex, Hungary*): By changing the ripping cut during flaying it is possible to obtain two bellies in one single piece and also two croupons in one piece. I think this idea comes from South France where they are following it for the last one year. But as far as I know, this is not followed in other parts of Europe. In northern part of Europe the hide is very thin in the croupons and is rather firm in the bellies and shoulder and has a very nice grain all over. So making croupon out of such hide is nothing but spoiling the hide. We make croupons out of 3 to 4% of our production and in Sweden, it may be a little more. I think, it must be a good idea to try but I am unable to tell you the results.

C. Halamek (*National Research Institute for Shoe, Leather and Allied Products, Gottwaldov, Czechoslovakia*) : By proper segmentation of the hide, it is possible to get a larger belly especially in the case of heavier hides. The most economic way of segmentation of the hide is to cut double shoulder as a single piece for making sole leather, double croupon and cutting the belly into two pieces taking care to see that the belly piece has a width of at least 21-22 cm and the total width of the croupon should not be less than 120 cm.

L. Schroeder : Such cutting of the hides into croupons, shoulder and belly may be possible and practicable in case of heavy hides.

P.S. Venkatachalam (*Central Leather Research Institute, Madras*) : May I know from Mr. Schroeder whether drum curing of hides and skins is done in his place?

L. Schroeder : No. We are not drum curing hides and skins. Regarding raw hides and skins we have to find out the cheapest and easiest way to prepare the hides for storing until they can go to the tanneries. If you put them in a drum and take them again, perhaps, they are salted in a slightly better way but I don't think we are doing it in Europe at all.

S. Bangaruswamy (*Central Leather Research Institute, Madras*) : Will Mr. Schroeder tell us the details about the integrated project 'warble fly campaign' as implemented in Denmark?

L. Schroeder : Denmark is a small country having only a short frontier down to Germany and is an ideal place to fight and get rid of warble flies. When the warbles were found on the cattle, the farmers had to inform the veterinary doctor. Besides taking the warbles out of the back of the animals, the affected cattle were held over in some isolated place. We are still now vigilant about this problem particularly at the German frontier.

Zille Singh (*Central Leather Research Institute, Madras*) : Majority of the hides in India are received from fallen animals and sometimes they reach the curers even after 3-4 days staling. Is there any simple and cheap method available to detect the actual staling period so that they can be treated with proper chemicals?

S.C. Nandy (*Central Leather Research Institute, Madras*) : This is a typical problem of of our country and not a common one in other countries. However, anyone who is directly dealing with this problem may probably give some suggestions.

P.L. Muthiah (*Central Leather Research Institute, Madras*) : In the case of fibres I have observed that after a time there won't be any further water absorption. Has Mr. Das studied water absorption at various intervals?

D.K. Das (*Central Leather Research Institute, Madras*) : The water absorption has been measured every half an hour upto a maximum of 3 hr. Maximum water absorption takes place within $2\frac{1}{2}$ hr in 90% of the hides. After that period there may be a slight increase but it is negligible. So we have decided to limit it to $2\frac{1}{2}$ hr.

S. Bangaruswamy : Besides determination of percent water absorption, density measurement would give additional information on the quality of leathers in which grain has drifted away from the corium. The density can be measured in less time and the method is simple.

Technical Session III

Ageing of Collagen

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The collagen molecule shows characteristic ageing. This can be shown by measuring the denaturation tension by heating in an isometric or isotonic arrangement. Similarly the socalled chemical contraction of collagen fibres with concentrated solutions of KI, $NaClO_4$ or urea may be used. Such measurements are only possible with collagen fibres from tendons. For other organs one may measure the solubility at denaturation temperature (65°C) and estimate hydroxyproline in the solution. With these methods, the biological age was tested with rat tail tendons or with skin. It was shown that the tension which tendon fibres produced at denaturation, differed according to the race of the animal. In rat, the tension of the tail tendon reaches 10 g at 30 months of age in cat 50 g at 15 years of age. It was found that if tendon fibres age *in vitro* that showed similar changes as *in vivo*.

Studies of water content related to this phenomenon showed that it was not simply the loss of water which caused an increased denaturation tension of stored fibres. With rapid evaporation of water at 32—55°C, the fibre was not aged for some time. Neither the increase in denaturation tension nor the decrease of solubility and swelling capacity changed for some time. It needed several days till these changes evolved. The physicochemical processes that may lead to these differences in time and whether these can be similar or identical to natural changes of ageing have been discussed.

It is well known that on denaturation, collagen fibres "shrink". Our first ageing test was therefore to measure the *thermal denaturation tension.* First we used an *isotonic* method as in general muscle-physiology[1]. Since, in the corium of the skin, the collagen fibres are not in an orderly direction, we used rat tail tendon (RTT) fibres. The maximal weight which can be lowered after heating to 60-70°C was used as an index of the maximal tension. The fiber thickness was 0.14 mm at 50 mm length and a weight of 2.5-3 mg

The older the animal, the larger was the maximal denaturation tension and the higher the temperature needed for the maximal contraction[2].

Isometric tension was measured[3] with a strain gauge ; this enables the determination of the maximal tension directly on a fiber.

When this maximal tension is attained the denatured fiber becomes glassy, transparent and elastic[4]. If one continues at the same temperature, relaxation follows. With isotonic method the fiber may come back to the original length[5]. With older fibers this depends not only on age but also on the weight and only larger weights bring it back to zero tension.

Complete relaxation can be achieved also by cooling the just denatured fiber to room temperature[6]. After relaxation such a fiber is unable to show a second thermal contraction.

If, however, cooling starts before reaching the maximal contraction, the elongated fiber can show a second contraction[7]. The sum of both is larger than the first one.

Thermal denaturation means a destruction of *H-bonds*. After the relaxation in the cold the second tension is possible because new H-bonds have been formed.

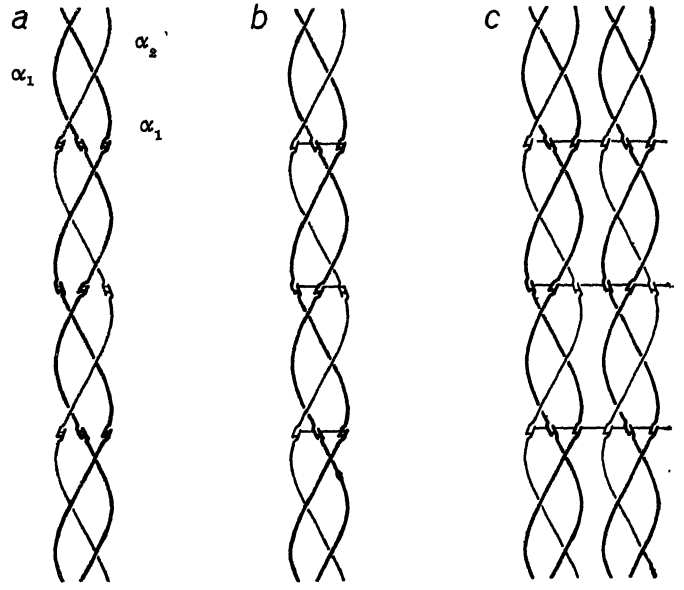

Fig. 1 *Crosslinks in Collagen (a) young (b) with intramolecular crosslinks (c) old with intermolecular crosslinks*

There is a second form of denaturation of collagen in which shrinking occurs, the so called *chemical contraction* with either 40% KI (Banga), 4M $NaClO_4$ (Chvapil) or 6 or 7M urea (Elden *et al*) etc. This action of only very concentrated solutions is generally explained as an extraction of water from the collagen fiber. Also this chemical contraction increases with age.

In explaining this, we may consider *H_2O-bonds* which are stabilising the helices of the collagen molecule. Their strength is larger than that of the H-bonds which figure in thermal denaturation. A superimposition of submaximal thermic contraction with chemical contraction is possible.

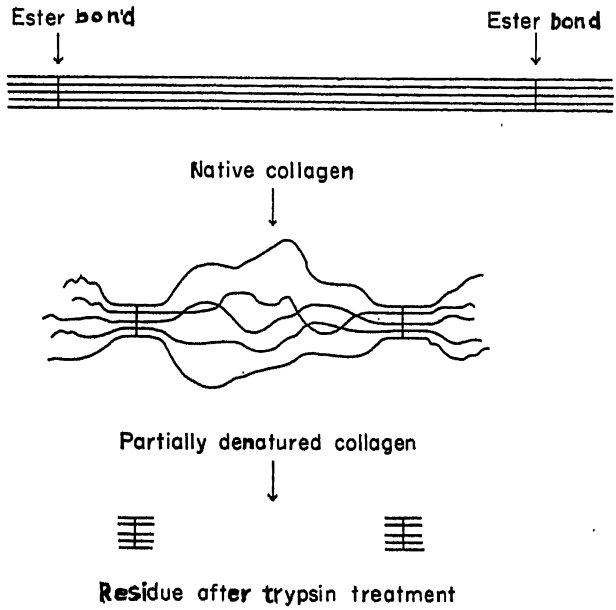

Fig. 2 *Native collagen partially denatured and finally digested* (After Horman).

Both the H-bonds and the H_2O-bonds counteract some other stronger bonds to which the shrinkage or the tension has to be referred. These third form of bonds are generally called *covalent bonds* and their nature is still unknown[8].

It should be mentioned that chemical contraction is partly reversible[9]. After washing out with water it can be repeated, but the effect is each time somewhat less. Each contraction liberates some hydroxyproline-containing collagen till finally no more hypro is released. Then the reversibility also ceases.

We have shown earlier that with each thermal denaturation *hydroxyproline* containing collagen goes into solution from the collagen fiber [10]. The quantity of this hydroxyproline is large in young animals and becomes less and less with ageing. Actually we measure collagen solubility at thermal denaturation. As is known from Piez *et al*[11] the dissolved collagen peptide has different qualities, namely α_1, α_2, β_1, β_2 and, δ collagen. This has not been followed till now with fibers of different ages.

Thermal denaturation liberates a part of the collagen. From skin or other organs of young animals, a large percentage of collagen is liberated and this quantity decreases with age.

Technically we used this method with organs on which no tension measurements are possible. We heat to 65°C, 10 min in Ringer's solution at *p*H 7.4. An estimation of total collagen is done by acid hydrolysis followed by hypro estimation and in a similar way the labile dissolved collagen is measured. The sum of both is the value of total, and the percentage the value of " labile collagen " which we use for characterisation of biological age.

This *hypro method* has been used to measure *biological age*[12] in many parenchym organs and especially in different parts of the skin, where no fibrillar tension can be measured.

Others have used different solubility methods to show changes of collagen with age but comparisons show that our hypro method after thermal denaturation is the best parallel to the *calendric* age of the animals. It can be also used for the skin of cattle or men.

Different parts of the skin give slightly different values. Skin of the back contains less labile collagen than that of the abdomen[13].

One interesting finding in men was that after a surgical cut the newly formed collagen of the scar is young collagen but in an old body. Thus, it is not that an old body produces old collagen. On the contrary new protein is always young[14].

The mechanical methods, isotonic and isometric tension measurements as well as the chemical hydroxyproline method are leading to the explanation, that with ageing stronger covalent bonds in the collagen molecule increase. The nature of these covalent bonds is still unknown and their chemistry will be discussed later.

We have to mention here that similar tension measurements as on RTT have been made also with tail tendon fibers of the cat[15] (CTT) which had the same diameter, length and weight as those of the rat. They can be isolated

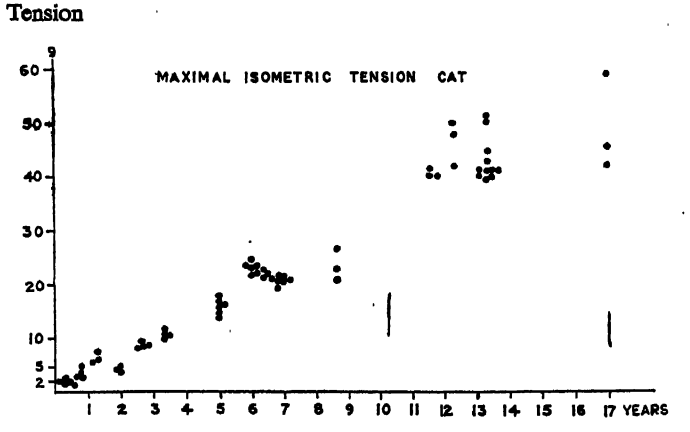

Fig. 3 *Isometric thermic-denaturation tension of cat tail tendon fibers of animals from 3 months to 17 years.*

from cat tail tendons similarly. The maximal tension in a rat of 3 years age is 9-10 g; in the 15 years old cats, it is 50 g. It is interesting, that at the maximal age of rats which is about 3 years, the tension is about the same as in 3 years old cat tendon. But since the cat lives 15 or more years, the collagen ages longer and the maximal tension becomes so much more. It is clear that the shorter lifetime of the rat has nothing to do with the ageing of its collagen.

Also in cold blooded animals collagen ages. We showed it in the frog[16] and *bowliers* in serpents[17].

The hypro method has been much used in rats, mice, men and in cattle and correlates well generally with the age of the animal.

The main gerontological problem is : what are the *covalent bonds* and why do they increase with ageing ? This has led to a search for tanning substances, which may be producing such covalent bonds. In the early forties a pathologist envisaged the arteries of old men to be stiff as if "tanned." Generally *Bjorksten* is associated with the idea that ageing is similar to tanning. One of the strongest tanning substances is formaldehyde. Aldehydes which appear in the metabolism of men and animals may become such crosslinking substances. Acetaldehyde is an example but it is present in such dilutions that it is improbable that collagen would be tanned by this.

The main difference between Bjorksten's view and ours is that he believes that globular proteins too become crosslinked in the aged. The crucial point of our work is that we underline that, only certain proteins are ageing, those which are not renewed during life. Collagen is the main example. After it is built up and deposited in the mature body, the collagen fiber is not renewed as we know from Neuberger[18] who marked with ^{14}C and from Thompson[19] who marked with ^{3}H Glycine the collagen produced in the young animal. This collagen will remain unchanged during the whole life : it has no "turn over."

We know now that, besides collagen, there are a few similar filamentous molecules which are not renewed : mainly *Deoxyribonucleicacid* (DNA)[20] of ganglion cells in the central nervous system, also in the nuclei of skeletal muscles and in the ovocytes which are not ripening. DNA is only renewed during mitosis. Cells which do not show a mitosis do not renew it. In all such cells it will age in a similar way as collagen. Both are helical filmentous proteins.

There is some proof that, in these mentioned cells, DNA ages. It becomes more stable, less souble. One possibility is that, as in collagen, covalent cross-links stabilise the molecule with increasing age. Collagen is our model for these processes, but the details of the ageing of DNA are still in discussion.

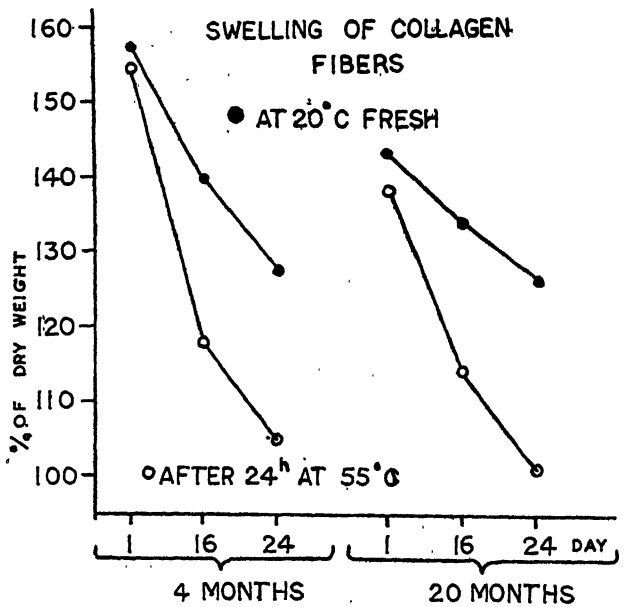

Fig. 4 *Swelling capacity of collagen fibers fresh and after at 55°C (rat tail tendon)*

The problem whether *collagen production* can be stimulated during life and especially during ageing has been discussed often. The collagen producing fibroblasts and fibrocytes are not definitely amitotic cells. They are able to function if certain stimuli act. It has been said that in old age, collagen production increases in general. I think this is a mistake. The fibroblasts are stimulated under certain conditions and we may ask what these conditions are?

We showed some years ago that in *wound healing* in the skin new collagen is produced in the corium and this is young collagen. It ages with the body as mentioned before. The stimulus for collagen production is the chang of tension in the tissue after wounding.

Another example on which we have worked is the question why the collagen increases. If one raises the tension of the skin by introducing a foreign body[20] under the back skin of the rat and makes an artificail bump, this increases the tension at that place and changes in collagen appear. In a certain distance new collagen is produced, as can be shown by changes of labile collagen, which is incraesed here. It must be of course mentioned that in the center, at very high pressure, collagen is destroyed and disappears, especially the young parts.

An even better example is the following. During *gravidity in the uterus* the quantity of collagen increases enormously about 6 times in the rat. In experiments with *Takacs* we have answered the question about the stimulus. Into the *double parted uterus* of a virgin rat one injects in one half insoluble parafin and extends it. A few days later this part contains much young collagen, while the other side does not. Thus increase of tension produces new collagen. Obviously fibrocytes start collagen production because the increase of tension is a stimulus.

The same mechanism seems to explain that in the growing rat the *abdominal skin*[21] is relatively younger than the skin on the back. Under the stimulus of the tension of the growing abdominal organs the skin is under tension and collagen production follows.

Also in the *ligamentum pubis* especially in the guinea pig, an intensive collagen production is introduced during gravidity, which also is "young" collagen.

Some confusion may have been raised by the description of a small increase of young collagen in *skeletal muscles* of ageing animals. This[22] is probably caused by the decreased tension which, in old muscles, appears

through the atrophy of muscle fibres. Similar facts may be present in other parenchymatous organs also[23].

Some work has been done on *hypophysectomized*[24] rats. We hypophysectomized the rats at the age of 6 weeks at about 200g body weight and growth was stopped. The animals became maximally 22 months old while normal rats lived about 35-40 months. The collagen of the hypophysectomized rats also ages, but is always relatively younger as in normals. Thus, ageing is independent of the pituitary, but it is diminished to a stage similar to that in a young animal. The ageing process seems to be retarded by a general metabolic decrease in these animals, and *not* accelerated, *as is said.*

From a gerontologist's point of view, the main problem is what the strong *covalent crosslinks* are, which increase with ageing and which characterise the aged collagen, make it stiff and decrease the adaptability to stress. Several laboratories work on this question. Horman[25] in Munchen believes that hexose sugar-bridges are formed which either as aldehyde or in ester bridges, give intra or intermolecular crosslinks. Seifter and Gallop[26] believe that amino acids are participating also, and that has been supported by others. It is suspected that mucopolysaccharides may be the origin for the carbohydrate, but this had not been proved.

This question has been touched in a rather unsuspected observation lately. I have always emphasised that fresh material should be used for

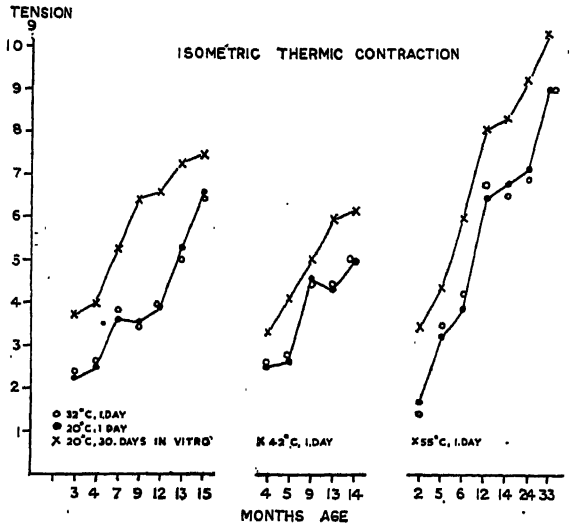

Fig. 5 *Isometric thermic denaturation tension of fresh and dried rat tail tendon*

tension experiments or for the hypro test; but others have studied tendon fibres kept several months *in vitro*. Arvay and Takacs[27] found that they show in the isotonic mechanical age test, an ageing similar to the ageing as in the body. Repetition of the experiments with isometric tension completely confirmed it. It is questionable whether the velocity is similar to the ageing *in vivo*. It may be quite accidentally similar for rats (which live only 3 years).

This change was supposed to be related to the *loss of water* after keeping the fibers *in vitro*[28]. To test this, we heated the fibers for 3 hr at 32°C or even to 55°C. Almost all the water is lost; thus at 20°C it requires 2-4 weeks. In spite of this, the tension changes appear not immediately, but only after 1-2 months. Similarly a decrease of denaturation *solubility* with the Hypro-test and a decrease of *swelling* capacity appear also only after some weeks. Thus, the low water content is not the cause of the increased tension at thermic denaturation which occurs only slowly.

Such slow reactions may be caused by new steric relations of the he ices inside the molecule which lead to very slow connections. However, it cannot be supposed that normal ageing is also caused by similar steric connections since the water content of collagen fibers decreases very little during ageing or not at all. The well known decrease of humidity in old age skin is caused by changes of the mucopolysaccharides.

It is much discussed at present whether water in collagen is present in a special crystalline state. Linus Pauling[29] was probably the first to notice that the crystalline state of water inside a cell could influence the reaction of this. He supposed that the narcotic capacity of different substances depends on the crystalline water structure inside ganglion cells of the central nervous system.

The state of water in collagen of different ages thas been measured by Berendsen[30] with nuclear magnetic resonance of collagen and he seems to have found certain characteristic differences. However, studies with Roentgen-rays diffraction as those of Narten *et al*[31] published lately in the Faraday symposia show that, even with purest water, water structure is not finally settled and, in the presence of other molecules, is probably more complicated. In the collagen it must interfere with the triple helix and, if cross-links are produced, the crystalline structure of water must be disturbed.

At present there is no explanation how water molecules stabilise the triple helix. Klotz[32] believes that water molecules also act by H-bonds and thermic denaturation also destroys them. The fact, thermic and chemical contraction tension can be added up, does not support this hypothesis.

Calcium may play a role as a crosslink in collagen under certain conditions. 10 years ago we showed that with age the affinity of collagen to ^{45}Ca increases[33]. It was also known that, with denaturation, the capacity of collagen to bind calcium increases, perhaps dicarboxylic acid residues of amino acids become free to which calcium may be bound.

As well known calcification, or as Selye[34] calls it "Calciphylaxis" can be introduced by intoxicating an animal with dehydrotachysterol (DHT). If some local stimulus, as $FeCl_3$ in dextran is injected subcutaneously localised calcification will follow. It has been shown by us that labile collagen decreases in these places and when even total collagen quantity is diminished. In the calcified tissue the relation of calcium and phosphor changes so that it is representative to apatite (10 : 6)[35].

Lansing[36] had shown an increase of calcium with age in the aorta, August in heart valves and Dalderup *et al*[37] in arteries in old animals.

The fact that under certain conditions, as mentioned for the case of calciphylaxis, collagen may decrease in the tissue, leads to the question under what conditions, *collagen may be destroyed* in the body.

It had been mentioned by Doloney and Pisano[38] and others that inflammation increases digestive activity of the polymorph nuclear leucocytes. Obviously their lysosomes liberate hydrolases which, then, can digest collagen under certain conditions. Also under conditions of sterile inflammation, the liberation of collagen destroying enzymes seems possible. Native collagen of old animals, which is stabilised by covalent bonds, is generally not or rather slowly d gested.

The following example shows conditions under which collegan is broken down. During *gravidity in the uterus* collagen increases enormously in the rat uterus, as mentioned, about 6 times. Immediately after gravidity all this collagen, which was newly produced, disappears completely in a few days[39]. This newly produced collagen is of course maximally 21 days old, since the rats' gravidity has this time. This newly produced young collagen has, therefore, very few or no covalent bonds and it is this collagen which is digested. After birth and after the placenta has been delivered, there follows a strong exudation and leucocytosis in the mucosa of the uterus. This is the picture of a sterile inflammation. The leucocytes deliver lysosomes and from these hydrolases. It is these, which digest the young collagen fibres. They do not act on the whole bodies collagen because that is relatively old and contains covalent bonds.

It may be mentioned that the same mechanism acts also on the newly formed collagen fibers in the *ligamentum pubis*[24] which is promptly destroyed after gravidity.

We started with the signs of ageing, with the description of *crosslinks* in collagen and their increase with age and methods, mechanical and chemical, to prove this increase. We then discussed what *stimuli* may cause the production of crosslinks in spite of ageing. Finally it is to be known, under what conditions, a *destruction* of collagen may be possible under biological and pathological conditions.

It was also discussed what the *covalent crosslinks* may be, which increase with ageing. Different *theories* were mentioned ; the role of *water* and the role of calcium have been discussed.

The main problems in the biology of collagen seem to be the question about the *nature of the covalent crosslinks* and of the role of water and inorganic substances and finally also of lysozymes in their action on collagen.

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Role of Normal Crosslinking Agents Present in the Body in Ageing Phenomenon

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The changes in the physical properties of body tissues on ageing were attributed to the crosslinking of the protein concerned by the accumulated metabolic intermediates, both organic and inorganic. The present investigations based on *in vitro* studies suggest that the metallic elements, Al, Fe and Si do not crosslink tissues similar to collagen at body *p*H but only aldehydes like pyruvaldehyde may.

In the present literature changes in the physical properties on ageing such as loss of elasticity, loss of ability to hold bound water, reduction in the capacity to swell, decreased susceptibility to proteolysis and an increased hydrothermal stability which are indicated by an increase in the shrinkage temperature of body tissue and fibrous proteins are attributed to the crosslinking of the protein concerned[1-4] by the progressive accumulation of potential crosslinking metabolic intermediates in a random like fashion. Potential crosslinking agents cited were both inorganic and organic. Aluminium, iron, silicon and zinc present in the body fluid are considered to be some of such inorganic impurities[1]. Among the organic class of compounds, some of the acidic and aldehydic intermediaries are mentioned. Keto glutaric acid, pyruvic acid, citirc acid, malic acid, succinic acid, glyceraldehyde, pyruvaldehyde, acetaldehyde, glyco-aldehyde, acrolein (vinyl aldehyde) are some such intermediaries[1-6]. Possibilities of the presence of other aldehyde like glyoxal, glutaraldehyde and certain other low molecular weight dialdehydes[3,5,6] and 0-catechol derivatives like quinone capable of crosslinking were also reported[1]. This work relates only to the

effect of mineral and aldehydic crosslinking agents on shrinkage. Among the other remaining set of impurities, organic acids like citric acid or citrates do not posses crosslinking characteristics on their own but they can complex and stabilise metallic mineral crosslinking agents of aluminium and iron (*e.g.* citrate is one of the well known complexing agents used in the preparation of basic aluminium sulphate). They crosslink collagen in acidic conditions generally in the *p*H range of 3.5 to 4.5 or 5 [7,8]. With the rise of *p*H, mineral tanning agents will precipitate unless proper masking agents in sufficient quantities are used. But it should be noted that the greater the masking, the lesser is the tanning action of aluminium or iron and hence rise in T_S is less if collagen is treated with such liquors. Based on this knowledge an attempt was made to find out whether metallic mineral impurities of the body, aluminium and iron, if properly masked can tan collagen at physiological *p*H (7.3) under *in vitro* condition.

Just as chrome, aluminium or iron, silicate, the non-metallic mineral tanning agent also tans collagen at acid *p*H (3.5). As in the case of aluminium and iron, experiments were carried out to find out whether silica tans Rat Tail Tendon collagen (RTT) directly at physiological *p*H.

As regards aldehyde impurities, it is to be noted that a good amount of work was done by Milch[8] and Gustavson[9]. Milch tanned bated goat pelt with various aldehydes, food metabolite intermediaries and others, and demonstrated the ability of various aldehydes except acetaledhyde to tan and raise its T_S. But in his experiment no proper attention was paid to complete the various aldehyde tannages at identical *p*H, the final *p*H of the bath varving from 4.1 to 7.4. Since aldehyde tannages are more susceptible to *p*H changes, it is imperative that they should be carried out and completed at identical *p*H, preferably at physiological *p*H for comparing and finding out the possibility and/or the extent of their ability to crosslink and this aspect was taken into consideration in the present work. Pyruvaldehyde and acetaldehyde, the two of the reported intermediary metabolites and formaldehyde were chosen.

Among the various chemicals considered for the present work only such of those which raise the T_S of collagen at *p*H 7.3 were further used for more detailed study of their effect on the shrinkage behaviour of collagen fibres. This was carried out by treating RTT with a solution of the concerned crosslinking agent of increasingly dilute concentrations and then noting its shrinkage characteristics T_S, Linear shrinkage (L.S) and Linear recovery (L.R.).

Material and Methods

Collagen fibre bundles freshly teased out from a one year old rat were placed in water and then treated overnight with various mineral tanning reagents such as Fe, Al, or Si at usual acidic tanning *p*H and physiological *p*H (7.3). Fibres were pickled with 1% sulphuric acid and 8% salt and used for tanning with aluminium, iron or silica in acidic *p*H. For tanning at physiological *p*H no such treatment was necessary and hence only raw collagen was used in such instances. While tanning RTT an infinite float was maintained so as to keep the *p*H and the concentration constant. T_s of the treated collagen fibres are determined by "micro" method (under publication).

It is to be noted that the T_s was determined at the normal tanning *p*H and physiological *p*H. In the case of physiological *p*H, T_s was also determined wherever necessary at *p*H 7.3 in sodium choloride solution containing a physiological concentration.

RTT was similarly treated with various aldehydes (1% solution) and the T_s values at the usual tanning *p*H (8.2) and 7.3 and in the presence of physiological sodium chloride are also given in Table 1. The effect of aldehydes at various dilutions on the increase of T_s, L.S. and L.R. was also studied.

Basic aluminium sulphate was prepared in the following ways :

(a) for treating at *p*H 7.3 : 100 g of aluminium sulphate was treated with 20 g of sodium citrate, moistened with 30 ml of water and heated to melt. To the melt was added 40 g of soda ash as 10% solution. After cooling, the *p*H of the solution was finally corrected to 7.3.

(b) for treating at *p*H 4.5 : The liquor was prepared in a similar manner using 10 g of citrate and 20 g of soda ash instead of the quantities mentioned above. As in the previous case, the *p*H was finally corrected to 4.5.

While treating RTT with basic aluminium salt solution a concentration of 1.5% Al_2O_3 was maintained in the liquor. To prepare basic iron salt solution 100 g of ferric sulphate was treated with 100 ml water and to this was added 20 ml of 10% soda ash which favoured complete dissolution. Then a 10% solution of a mixture of soda ash and sodium citrate (1 : 1) was added in drops with vigorous stirring till the *p*H was raised to 4.5. At *p*H 4.5 half of the solution was removed and suitably diluted so as to have a Fe_2O_3 concentration of 1.5% for tanning RTT. The remaining half was further treated with citrate and soda ash in the aforesaid manner till the *p*H reached 7.3.

This liquor after suitably diluting (1.5% Fe_2O_3) was used for treating RTT. It is to be noted that both Al and Fe basic liquors were aged for 1 week before using them for tanning.

The *p*H of 1% sodium silicate soultion was decreased by dilute HCl. with good stirring for treatment with silica at *p*H 7.3 but it was observed that the liquor jelled completely at *p*H 7.3. Hence, fresh RTT was treated overnight at room temperature with the silica solution of 0.1% concentration at *p*H 7.3.

For comparison, pickled RTT was treated with sodium silicate solution (1%) very slowly, in drops with constant stirring. As soon as the gelation took place in the tanning bath, addition of silica was stopped. It is to be noted that the time taken for raising the *p*H of the pickle bath by sodium silicate was 6 hr. Such a slow rate of addition was necessary to avoid "case hardening" caused by the deposition of silica on the surface of the fibres.

Results and Discussion

It can be seen from Table 1 that Al, Fe and Si raise the T_S of collagen on tanning at fairly or highly acidic *p*H but do not raise the T_S at physiological *p*H, irrespective of whether T_S was determined in water or in the sodium

TABLE 1

Shrinkage temperature of collagen fibres treated with mineral and other organic impurities at physiological and their respective normal tanning pH

Treatment	Usuaul tanning pH	Concentration of solution used	T_S°C		
			Usual tanning *p*H	Physiological *p*H	Physiological *p*H and physiological NaCl
Al as basic aluminium sulphate (a)	4.5	10%	74 67*	59*	60*
Fe as basic ferric sulphate (b)	4.5	10%	72	60*	59*
Mixture of (a) and (b)	4.5	Fe 5% Al 5%	71	59*	60*
Si as silicate	3.0	1%	85	58*	58*
Formalin (HCHO 40%)	8.2	1%	88	86	86
Pyruvaldehyde	8.2	1%	74	71	70
Acetaldehyde	8.2	1%	64	65	64

*Liquor containing extra amount of masking salt so that the liquor is stable even at *p*H 7 to 7.3

chloride solution. In the case of aldehydes, however, there is a rise of T_s at *p*H 7.3 in the presence or abesence of sodium chloride in physiological concentration. It may also be seen that if the *p*H of the tanning bath is

Raw RTT	T_s°C	58
(Control)	L.S.%	70
	L.R.%	14

RTT in 1% formalin at *p*H 4.5 T_s°C 74

maintained at 4.5 instead of at 8.2 the T_s of formaldehyde treated collagen is lowered by 14°C, a significant decrease indicating the importance of *p*H in aldehyde tannages. Since rise of T_s is an indication of crosslinking and the extent of rise in T_s is an indication of strength of such links it may be said that only aldehydes getting accumulated in the body is responsible for rise in T_s of tissues of aged being. But in view of Zinsser's[10] observation based on X-ray studies that Al is present in aorta tissues, it may be said that under *in vivo* condition some other mechanism responsible for crosslinking is probably operative.

In addition there are certain regions in the body where the tissues come into contact with acidic secretions *e.g.*, stomach and to some extent "duodenum". Hence these regions are more prone to crossslinking by mineral impurities. But this again is rather rare possibility because of the fact that mineral crosslinking agents like basic aluminium salts are not stable at a high dilution and get precipitated. Moreover, if such possibility exists the stomach or duodenum of those who consume quite a good quantity of antacid drugs based on aluminium should have been completely tanned resulting in a premature death of the individual. But it is not so.

Results given in Table 1 indicate that among the aldehydes studied, rise in T_s due to acetaldehyde tannage at *p*H 7.3 or 8.2 is only 4°C whereas rise in T_s due to HCHO tannage under identical condition is quite phenomenal. Pyruvaldehyde is again quite a good crosslinking agent.

The relation between T_s and concentration of aldehyde used for tanning indicates that only aldehydes like formaldehyde which can form crosslinks like methylene bridges can raise the T_s effectively and in such instances recovery is also good. In other instances, *e.g.*, acetaldehyde or pyruvaldehyde treatment rise in T_s is not phenomenal even at higher concentration and recovery is also poor. Using formaldehyde at very high dilution 10^{-5} or 10^{-6} g/ml concentration the shrinkage values of treated RTT corresponded

to that of raw collagen. These results indicate that if aldehydes are fixed unipointly as in the case of acetaldehyde, the rise in T_s will be poor. This perhaps gives one of the explanations, though indirectly to the cause of quick death of methanol poisoned individuals compared to the slow lowering of life expectancy of ethanol addicts.

It is to be noted in this connection that when alcohols are consumed they are first oxidised by alcohol dehydrogenase into respective aldehydes[11]. Since aldehydes are fixed in alkaline conditions, they mainly attack the liver, pancreas or kidney[12] and such other glands which produce alkaline secretions with the result that these will become rigid, because of tanning and their activity is affected. Since acetaldehyde, as mentioned earlier, can fix only unipointly, the activity of such glands are frequently and temporarily affected as and when they come into contact with acetaldehyde due to the consumption of alcoholic beverages. But in the case of methanol, formaldehyde formed tans and makes the concerned tissue irreversibly stiff and hard and hence the functions of various glands are heavily damaged. The above statement needs confirmation by study *in vivo*.

The possible greater hardening of relevant body tissues by methanol poisoning when compared to ethanol ingestion as a consequence of the action of respective aldehydes formed is substantiated by the fact that the wet average compressibility* of thirty acetaldehyde treated delimed pelt pieces is 70% and the average value of those treated with formaldehyde is 45%, the treatment being done under identical conditions. This along with the shrinkage characteristics indicates that the ageing will be accelerated by the greater accumulation of more powerful crosslinking agents in the body.

It is apprent that the T_s and L.R. values (Table 2) decrease with the lowering of formaldehyde content which is due to infinitesimally small ability of this aldehyde to crosslink at very low concentration. Hence the rise in T_s

*For measuring the wet compressibility of aldehyde treated pelts, adjacent pieces of delimed goat pelt samples, prepared by the procedure adopted by us in our earlier work[13], were treated, after a thorough wash to remove the adhering deliming chemical, for 8 hr with equimolar solution of acetaldehyde and formaldehyde maintaining a float of 800% to the pelt and a *p*H of 7.3. Percent compressibility was determined by noting the initial reading with the sample beneath the plunger of the C & R tester (Custom Scientific Instrument Corpn. Inc., Kearny, N.J. CS-55-021) and the final reading with the sample beneath the presser foot was subjected to a particular load. The presser foot used is a circular flat disc of 2.9 cm diameter and a load of 747.5 g (26 oz) was applied on it for noting the final reading when the sample underneath was compressed. Since the skin-samples were wet and as there was a good amount of variation in value due to interference of water the average percent compressibility indicates only a gross trend.

TABLE 2

Relation between shrinkage characteristics of collagen treated with aldehydes and their concentration at physiological pH

Aldehyde	Shrinkage values	Concentration				
		1×10^{-3}	1×10^{-3}	1×10^{-4}	1×10^{-5}	1×10^{-6}
Pyruvaldehyde	T_S°C	71	68	65	62	59
	L.S.%	75	76	72	70	—
	L.R.%	34	17	16	19	—
Acetaldehyde	T_S°C	65	62	—	—	—
	L.S.%	75	—	—	—	—
	L.R.%	20	14	—	—	—
Formalin (aldehyde content 40%)	T_S°C	85	77	68	62	59
	L.S.%	72	72	76	70	70
	L.R.%	55	59	24	15	13

of collagen on ageing used is not very high and the small concentration of aldehyde used is not sufficient to bring about a perceptible recovery of shrunken collagen even when the collagen is from an aged animal.

Thus, these data go to suggest that on ageing, aldehydic impurities getting accumulated in the body and not the mineral impurities are responsible for the rise in T_S and accompanied physical changes of the body tissues. However, it is to be noted that the present study is under conditions *in vitro*. For complete interpretation there are other factors like phase equilibrium and membrane transport which have to be taken into account.

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Effect of Different Levels of Dietary Protein on Collagen and Nucleic Acids Content of Albino Rat Skins

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With a view to study the changes in collagen and nucleic acids content of skins due to the influence of different levels of dietary protein, four groups of weanling albino rats were fed with control, high protein, low protein and protein-free diets for three weeks and glycine-1-C^{14} was injected to each rat. The analyses of skins, urine and plasma showed that the total collagen, neutral salt-soluble collagen, free glycine and RNA content of skins, the incorporation of glycine-1-C^{14} into skin collagens, the urinary excretion of both bound and free hydroxyproline and the free hydroxyproline content of plasma were all significantly reduced in the low protein and protein-free groups compared to the control group whereas there was practically no change in any of these characteristics in the control and high protein groups. These results collectively show that the synthesis of skin collagen was considerably decreased in the low protein and protein-free groups compared to the control group whereas the increase in the dietary protein level more than the adequate quantity did not increase the synthesis of skin collagen.

It was reported[1] that the total body protein was lower in the malnourished children fed on a low protein diet than in normal children. The rate of formation of collagen in the animal body was found[2,3] to be decreased owing to protein deficiency. Tryptophan deficiency also caused a reduction in skin collagen synthesis[4,5]. Delayed or impaired healing in skin wounds of protein deficient animals was also reported[6-12]. Depending on the growth rate of animals, the quantity of neutral salt-soluble collagen of skin was found[13-16] to vary. It was also reported[17-21] that the ribonucleic acid (RNA) content of liver increased or decreased with the nutritive value and the rise or fall of protein intake but the deoxyribonucleic acid (DNA) content of

liver was not affected by protein deficiency. So far no report is available with regard to the changes in nucleic acids content of skins due to the influence of the level of dietary protein.

Since hydroxyproline is present in high concentration only in collagen, the excretion of this imino acid has been taken[22,23] as a parameter for evaluating collagen metabolism. The urinary hydroxyproline excretion is related to the rate of growth, collagen metabolism and the amount of soluble collagen in the animal[24-30]. The amount of free hydroxyproline in plasma also reflects the changes in collagen metabolism. The main object of the present investigation is to compare the effects of protein-free, low or high protein diets on the collagen content of albino rat skins as measured by the skin hydroxyproline content, the incorporation of labelled glycine into skin collagen, free glycine content of the skin, the amount of neutral salt-soluable collagen of skin, the rate of excretion of hydroxyproline in urine and the plasma hydroxyproline level. The changes in RNA and DNA contents of the skins due to the influence of protein-free, low or high protein diets have also been investigated.

Materials and Methods

Four groups (24 each) of weanling albino rats were raised on control diet (20% protein), high (55%) protein, low (6%) protein and protein-free diets, the details of which were described in a previous communication[31]. Casein which is known to have high biological value was used in these diets. They were fed *ad lib* for 21 days with the respective diets and on the last day the urine from each group was collected under toluene for a period of 24 hr After collection of urine, glycine-1-C^{14} was injected to each rat intraperitoneally, the dose injected was 10 μc/100 g body weight. Five hours after injecting the labelled glycine, the rats were killed by decapitation and the blood sample from each group was collected in presence of an anticoagulant and the plasma was separated immediately. The skin was removed from each rat, unhaired with a razor blade and fleshed. The skins from each group were pooled together for analyses.

Total collagen :- As the determination of the amount of hydroxyproline which is a characteristic amino acid of collagen, has been used[32,33] as a measure of the collagen content of tissues, the hydroxyproline content of the skins was estimated. An aliquot of the skins was cut into small pieces, dehydrated in acetone, defatted with light petroleum ether and air dried. The dried material was hydrolysed with 6N HCl in an evacuated and sealed tube at 110°C for 24 hr. After the removal of HCl, the hydroxyproline content of each

skin hydrolysate was determined by the method of Prockop and Udenfriend[34]. The collagen content of the skins was calculated by multiplying the hydroxyproline content by the factor 7.46[35]. As elastin also contains about 1.6% hydroxyproline[36] and a small amount of elastin is present in the skin, the results on collagen content when calculated from the hydroxyproline content of skins will be slightly higher. This small error is considered negligible for a comparative study of this type.

Neutral salt-soluble collagen :- Another aliquot of the skins was finely minced in a meat mincer and the macerated material was extracted[37] thrice by shaking for 24 hr at 4°C with 5 volumes (V/W) of 1 M Nacl at *p*H 7.4 using octan-2-ol as a preservative. The combined extract was dialysed, hydrolysed and the hydroxyproline content of the hydrolysate was determined[34] and the collagen content was calculated as mentioned before. The values were expressed as hydroxyproline or collagen per 100 g dry defatted skin.

Radioactivity of gelatin:- For the measurement of radioactivity, the collagen should be prepared in pure form, because any contamination of non-collagenous proteins will affect the results. Hence the collagen from the skin of each group was prepared as purified gelatin by the method of Jackson[38] The gelatin was hydrolysed and the hydrolystate was converted to DNP-amino acids by the method of Sanger[39]. After removing the dinitrophenols by the cold finger sublimation technique of Mills[40], the DNP glycine was isolated by the method of Perrone[41] as described by Neuberger *et al*[42] and its radioactivity was measured in a Geiger-Muller Counter at infinite thinness by the procedure of Henriques *et al*[43]. The values for radioactivity were expressed as specific activity (counts per minute per mg of gelatin).

Free glycine content of skin :- In order to check up whether the decreased incorporation of labelled glycine into skin collagen of protein-free and low protein groups was due to the increase in the glycine precursor pool and to the greater dilution of the injected labelled glycine, the free glycine content of the skins of each group was determined. An aliquot of the skins was homogenised, the protein-free filtrate of the homogenate was prepared by the tungstic acid procedure and the glycine content of the protein-free filtrate was determined by the method of Alexander *et al*[44] as adopted by Smith and Allison[45]. The values were expressed as mg glycine per 100 g dry defatted skin.

RNA and DNA contents of skin :- The RNA and DNA contents of the skins were calculated from the ribose and deoxyribose contents which were estimated by the methods of Ceriotti[46,47] as adopted by Natarajan and Bose[48]. The values were expressed as mg RNA or DNA per 100 g dry defatted skin.

Analysis of Urine and Plasma :-. The total and free hydroxyproline contents of urine or plasma were determined upon hydrolysed and unhydrolysed samples respectively by the method of Prockop and Udenfriend[34] and the bound hydroxyproline content was calculated in each case.

Results and Discussion

The results obtained on the changes in total collagen and neutral salt-soluble collagen contents of skins due to the influence of different levels of dietary protein are presented in Table 1. Compared to the control group,

TABLE 1

*Hydroxyproline, total collagen and neutral salt-soluble collagen contents of skins of albino rats fed with the different diets**

Group	Skin			IM NaCl extractable fraction of skin		
	Total hydroxy-proline	Total collagen	% decrease in total collagen compared to control	Hydro-xyproline	Neutral salt soluble collagen	% decrease in neutral salt soluble collagen compared to control
Control	10.77	80.35	...	0.559	4.17	...
Protein-free	7.11	53.04	33.99	0.076	0.57	86.34
Low protein	8.16	60.87	24.24	0.257	1.92	53.96
High protein	10.62	79.21	1.42	0.552	4.12	1.20

*Expressed as g/100g dry defatted skin

the total collagen content of skins of low protein and protein-free groups was found to decrease by 21 and 34% respectively. Much greater reduction (54 and 86%) in the neutral salt-soluble collagen content of skins was observed in the low protein and protein-free groups respectively compared to the control group. Significant differences were not observed in the total collagen contents or in the neutral salt soluble collagen contents of skins from high protein and control groups.

The results regarding the effect of different levels of dietary protein on the radioactivity of gelatins and free glycine content of skins are shown in Table 2. Significant decrease (52% and 74%) in the specific activity of

TABLE 2

Specific activity of gelatins and free glycine content of skins of albino rats fed with the different diets

Group	Specific activity (Cpm/mg gelatin)	% decrease in specific activity of gelatins compared to control	Free glycine (mg/100 g dry defatted skin)	% decrease in free glycine content of skins compared to control
Control	105.1	—	148.5	—
Protein-free	27.7	73.64	107.8	27.40
Low protein	50.6	51.86	124.0	16.50
High protein	104.9	0.19	148.2	0.20

skin gelatin after the injection of glycine-1-C^{14} was obeserved in the low protein and protein-free groups respectively compared to the control group. The free glycine content of skins of low protein and protein-free groups was also found to decrease by 17 and 27% respectively compared to the control group. No change, however, was observed in the specific activity of skin gelatins or in the free glycine content of skins of control and high protein groups.

TABLE 3

Nucleic acids contents of skins of albino rats fed with different diets

Group	Nucleic acids (mg/100g dry defatted skin)		% decrease in RNA compared to control
	RNA	DNA	
Control	558	153	...
Protein-free	178	150	68.11
Low protein	382	152	31.54
High protein	558	154	0

Table 3 shows the RNA and DNA contents of skins of control, protein-free, low and high protein groups. Significant reduction (32 and 68%) in the RNA content of skins was observed in the low protein and protein-free groups respectively compared to the control group. The RNA content of skins of control and high protein groups was found to be the same. There was practically no change in the DNA content of the skins due to the influence of the level of dietary protein.

Table 4 shows the effect of different levels of dietary protein on the urinary hydroxyproline excretion and plasma hydroxyproline content in albino rats. Compared to the control group, the urinary excretion of bound hydroxyproline decreased by 32 and 69% in the low protein and protein-free groups

TABLE 4

The urinary hydroxyproline excretion and the plasma hydroxyproline content in albino rats fed with the different diets

Group	Urinary hydroxyproline μg/24 hr		Hydroxyproline content of plasma μg/ml	
	Free	Bound	Free	Bound
Control	78.6	406.8	12.1	20.7
Protein-free	52.1 (—33.71)	126.5 (—68.92)	8.2 (—32.23)	21.2 (+ 2.41)
Low protein	68.7 (—12.60)	276.3 (—32.07)	9.8 (—19.01)	21.0 (+ 1.45)
High protein	79.2 (+ 0.76)	410.8 (+ 0.98)	12.3 (+ 1.65)	20.6 (— 0.48)

The values in parentheses show the percentage decrease or increase compared to control.

respectively. Much lower reduction (13 and 34%) in the urinary excretion of free hydroxyproline was observed in the low protein and protein-free groups respectively compared to the control group. There was no major change in the urinary excretion of bound or free hydroxyproline in the high protein and control groups. In the case of plasma, however, only the free hydroxyproline content was found to decrease by 19 and 32% in the low portein and protein-free groups respectively compared to the control group. The bound hydroxyproline content of plasma was not affected due to the influence of the level of dietary protein. There was also no change in the free hydroxyproline content of plasma in the high protein and control groups.

Significant reduction in the neutral salt-soluble collagen and also in the total collagen contents of skins was observed in the low protein and protein-free groups compared to the control or high protein groups. Very little amount of neutral salt-soluble collagen could be extracted from the skins of protein-free groups. The fraction extracted with neutral salt solution may

be considered to containt he most recently synthesised collagen molecules[49]. The specific activity of skin collagen after the injection of glycine-1-C^{14} was appreciably decreased in the low protein and protein-free groups compared to the control or high protein group. These results collectively show that the synthesis of skin collagen was greatly reduced in the low protein and protein-free groups.

The decreased incorporation of labelled glycine into the skin collagen may also result if the glycine precursor pool was increased. It was, however, observed that the free glycine content of skins of low protein and protein-free groups was considerably decreased. It is, therefore, evident that the decreased incorporation of glycine-1-C^{14} into the skin collagen of low protein and protein-free groups was mainly due to the decreased synthesis of skin collagens.

Significant reduction in the urinary excretion of both bound and free hydroxyproline and the free hydroxyproline content of plasma in the low protein and protein-free groups may also be due to decreased synthesis of collagen and may not be due to collagen breakdown, as collagen catabolism would increase the urinary excretion of hydroxy-proline[28,50-54]. Appreciable reduction in the growth rate[31] of the rats and in the neutral salt-soluble collagen and also the total collagen contents (Table 1) of skins was observed in the low protein and protein-free groups. Recent studies with ^{14}C-proline showed that in young rats considerable proportion of the urinary hydroxyproline is derived from the soluble collagen fractions, whereas in older animals relatively greater amounts of urinary hydroxyproline are derived from catabolism of the insoluble collagen fibres[55,56]. It, therefore, appears that atleast in young animals the lower urinary hydroxyproline excretion indicates decreased collagen synthesis. As practically no change was observed in the total collagen, neutral salt-soluble collagen, free glycine or RNA contents of skin, in the specific activity of skin collagen, in the urinary excretion of hydroxyproline or in the free hydroxyproline content of plasma of control and high protein groups, it indicates that the higher level of dietary protein over and above the adequate quantity needed by the animal body did not increase the synthesis of collagen in skins.

It was also observed that the RNA content of skins of low protein and protein-free groups was much lower compared to the control or high protein groups. Spiegelman *et al*[57] and Paradee[58] suggested that, for the synthesis of protein, new RNA is synthesised concomitantly. Springell[59] found labelled collagen associated with RNA in sheep skins. Hosoda[60] reported an intimate relationship between soluble collagen and nucleic acids in the regene-

rating skin wound. Collagen differs from many other proteins with respect to its biosynthesis and also the content of its characteristic amino acids viz., hydroxyproline and hydroxylysine which are not derived from the corresponding free amino acids. Manner and Gould[61] and Daughaday and Mariz[62] suggested that soluble RNA (s-RNA) hydroxyproline, formed by the hydroxylation of s-RNA proline, is an intermediate in the incorporation of hydroxyproline in collagen biosynthesis. Several other[63-66] workers also provided evidence for the existence of hydroxyprolyl s-RNA in skin. It, therefore, appears that RNA plays a major role in the hydroxylation of proline and lysine in collagen synthesis in skins.

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Investigation on Collagen Degradation by Irradiation

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Samples of hide powder were subjected to ultraviolet irradiation for different known periods. The samples thus irradiated were tanned with different tanning agents. It was found that the uptake of tanning materials by irradiated samples increases progressively with the irradiation period indicating thereby the increase in the number of bonding sites. The possible mechanism of enhanced uptake appears to be due to the breakage of compensated hydrogen bonds and making additional bond forming sites due to irradiation.

A watersoluble fraction was obtained from the UV irradiated hide powder which was otherwise an insoluble material. Some chemical properties of the irradiated samples and their watersoluble fractions have been studied. X-ray diffraction study of the samples has also been made.

Studies on the effect of the radiations on the structure and properties of fibrous proteins in general [1-4] and collagen and leather in particular have already been made by various workers. Most of these investigations were concerned with the proteins in solution. Information regarding the effect of radiations on proteins in dry state is rather scanty. The problem of the effect of radiations on biological materials in general, and collagen in particular has of late assumed a great importance in view of frequent nuclear-explosions and space exploration activities. The fact that collagenous materials are continuously exposed to UV radiations from the sun makes it more imperative to conduct such study in an exhaustive manner with collagens from various sources. Such studies with UV as well as other radiations, it is expected, may help in identifying the specific or active sites in collagen chain susceptible to radiations and also to throw some light on the mechanism of the process of degradation. The present paper deals with the effect of UV

radiations on collagen in dry condition, the source being the hide powder. The results are expected to be of value in devising methods for making leathers and collagenous materials more resistant to radiation environments.

Materials and Methods

The source of collagen in the present investigation is standard hide powder. Ultraviolet radiation from a Philips Mercury ultraviolet lamp was used. The hide powder samples were spread in petri dishes kept at a distance of 30 cm apart to get an exposure. The samples were irradiated for 5, 10, 15, 20 and 25 hr. Tannic acid, mimosa, sumac and sulphited quebracho were employed for tanning the control as well as the irradiated samples. Tanning was done for 2 hr in a mechanical shaker and kept overnight. Next day, they were run for another 2 hr. The samples thus tanned were allowed to dry in air. They were next analysed for fixed tannin contents. Nitrogen estimations of the samples irradiated for different periods and also of their water extracts were made by the Kjeldahl method. The results were compared with identical determinations of the non-irradiated samples.

Wide angle X-ray powder photographs of the control and the hide powders UV irradiated for different periods were taken with the help of a flat camera using CuKα radiation.

Shrinkage temperature was determined by a micro-shrinkage meter in water medium.

Results and Discussion

TABLE 1

Fixation study with tanning materials

Time of irradiation (hr)	g of tannin irreversibly fixed by 100 g hide powder			
	Tannic acid (tanned at *p*H3.2)	Mimosa (tanned at *p*H4.2)	Sumac (tanned at *p*H3.8)	Sulphited quebracho (tanned at *p*H5.4)
Control (Non-irradiated)	45.72	43.48	26.42	44.65
5	45.86	44.65	26.50	45.72
10	48.38	47.25	28.26	48.28
15	50.26	48.20	29.66	50.32
20	53.78	50.64	29.62	53.46
25	54.65	52.72	30.12	56.72

Table 1 shows the results of the fixation study of the hide powder irradiated for different periods together with the non-irradiated control sample with some tanning materials. It can be seen that the percentage of the tannin fixed by the irradiated samples gradually increases with the irradiation period. When compared with the non-irradiated hide powder, the difference in the grams of tannin fixed by 100 g of hide powder irradiated for 25 hr is appreciable.

The nitrogen content (g/100 g of sample) of the hide powder irradiated control sample and the shrinkage temperatures in water medium for these samples are presented in Table 2. It can be seen that nitrogen content diminishes with the irradiation period with a parallel decrease in the shrinkage temperature.

TABLE 2

Nitrogen content and shrinkage temperature of the control and UV *irradiated hide powder*

	Control hide powder	Time of irradiation				
		5 hr	10 hr	15 hr	20 hr	25 hr
%Nitrogen	18.28	18.18	18.12	17.92	17.85	17.68
Shrinkage temperature (in water medium)	44°C	42°C	40°C	37°C	36°C	35°C

Table 3 shows the nitrogen content of the portion of the hide powder made soluble in water after irradiation for different periods. It is clear from the table that some substances containing nitrogen are rendered soluble in water after irradiation, which were otherwise insoluble in the same solvent. It seems that certain amino acids might be susceptible to UV radiations and the peptide bonds adjoining these residues may split and come in the water extract.

TABLE 3

Nitrogen content in the water extract of the irradiated hide powder

Time of irradiation (hr)	Percent soluble of total nitrogen
Control (non-irradiated)	—
5	5.08
10	7.72
15	9.48
20	10.50
25	10.75

Fixation study of tannins by collagen throws light on the bondforming sites in collagen. It has been suggested by Gustavson[5] that the vegetable

tannins mainly fix on the peptide bonds which are not internally compensated by hydrogen bonds, and also perhaps on other groups particularly basic ones such as ε – amino groups of lysine, guanidyl groups of arginine and the carboxyl groups of glutamic and aspartic acids. Results on fixation study with

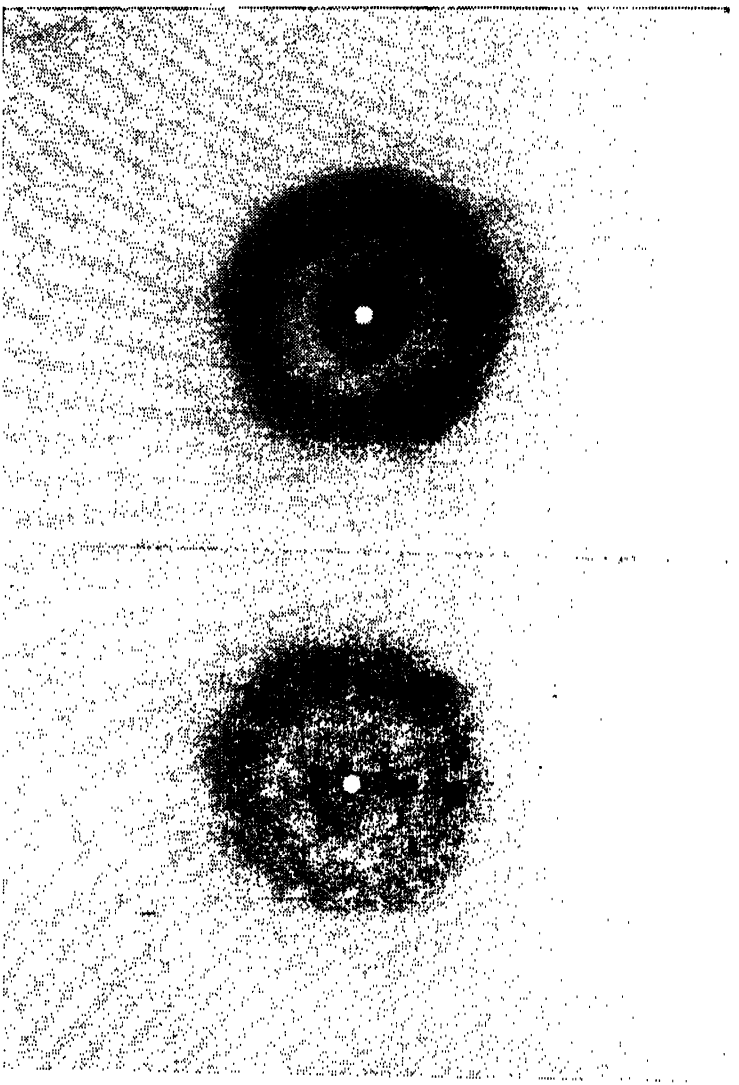

Fig. 1 (Top) *Wide angle X-ray photograph of hide powder (non-irradiated)*

Fig. 2 (Bottom) *Wide angle X-ray photograph of hide powder irradiated for 25 hr*

different tanning agents for the control as well as the irradiated hide powder for different periods of time showed that the percentage of irreversibly fixed tannins increased gradually with the increase of irradiation period. Thus the increase in fixation of tannins by irradiated hide powder may be due to the participation of some new bonding sites, formed mostly in the —CO.NH— groups which were internally compensated by hydrogen bonds before irradiation. Since hydrogen bonds are somewhat responsible for the orderliness of fibrous structure of collagen, some disorder in the structure is expected due to irradiation. A comparison of X-ray photograph due to normal hide powder (Fig. 1) and those due to irradiated sample (Fig. 2) for different periods corroborates this view.

If the stability of the fibre is somewhat reduced by the breaking of some hydrogen bonds with the irradiation, then less energy will be required to force the polypeptide chains apart and hence the shrinkage temperature should be reduced. This is also in agreement with the results shown in Table 2.

The decrease in the percentage of nitrogen from a value of 18.3 in the non-irradiated hide powder to a value of 17.7 in the 25 hr irradiated sample may be due to some loss of amide groups in the protein. The gradual increase of nitrogenous materials in the water extracts of the samples irradiated for different periods showed that some breakdown products due to irradiation are coming into solution, which were otherwise insoluble. It is known[5] that amino acids containing the aromatic ring e.g. phenylalanine, tyrosine and tryptophan are susceptible to UV radiations and the peptide bonds in the protein adjoining these residues may split up and come into the water extract. The study on the chemical nature of the water extract is in progress.

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Studies on the Variations in the Band Patterns of Collagen Fibrils from Different Sources

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Stained collagen fibrils obtained from mammalian, avian, reptilian and other sources were examined in the electron microscope and the band patterns were analysed. The results showed that the relative intensities of the cross striations varied with the source. The possible influence of the composition and sequence of the amino acids and the presence of the non-collagenous components on the intensity distribution of the band patterns is discussed.

Species variations in the structure and properties of proteins are important from a phylogenic point of view and such differences are widely reported in literature. Thus, to mention only a few, cytochrome C[1], somatotropins[2], serum proteins[3], haemoglobin[4] and insulin[5] exhibit important differences in their composition and properties with species. Collagen is no exception to the general rule and diversities in shrinkage temperature[6], chemical composition[7], physical properties[8] and crosslinkages[9] are well known. Collagen has been found to occur in a wide range of species and much remains to be done to understand the part played by evolution in this protein.

A few differences in the electron microscopical appearance of collagen have also been noted[10,14]. With the available refinements in specific electron stains, finer details in the band patterns could be made visible. The

occurrence of fine structure was attributed by Wolpers[15] to the biological state of the tissue and was considered to arise, in part, from abnormalities indicating structural degeneration. But it is now well established that the presence of a very detailed intraperiod fine structure in collagen fibrils arises out of a lateral alignment of the characteristic amino acid residue sequences in parallel chains. Since the amino acid content[8] (especially of the polar amino acids) varies with the source and since these are responsible for the intensity in the cross-striations of collagen fibrils observed in electron micrographs, a study on these lines was undertaken to get inrformation on the fine structure of collagen fibrils.

Materials and Methods

Achilles tendon of the horse, the neck tendon of dove, the tendon body of a rat snake and the fin rays of an unidentified species of shark were used as sources of collagen fibrils. Collagen fibres were teased from the tendons soon after the death of the animal and were purified according to the method of Bergmann and Stein[16]. A few fibres from each source were disintegrated in a hand-operated glass tissue homogeniser. Drops of the cloudy suspension of fibrils were dialysed over formvar films. The samples, mounted on copper specimen grids, were dried and stained with uranyl acetate at a *p*H of 4.0. The excess stain was removed by filter paper. The concentration of the stain was 1%. The samples were scanned in a Siemens Elmiskop I.

Results and Discussion

The intraperiod band pattern of the collagen fibrils showed characteristic differences depending on the source from which they were obtained. The shark ray collagen (elastoidin) occurred in sheet-like structures, unlike other collagen fibrils, which had circular cross-section. Within a period, three intrabands could be observed on staining. Even at high magnifications (Fig. 1) attempts to resolve a larger number of sub-bands were unsuccessful[14,17]. On the contrary, a larger number of fine bands could be discerned in collagen fibrils obtained from rat snake (Fig. 2), dove (Fig. 3) and horse (Fig. 4) tendons.

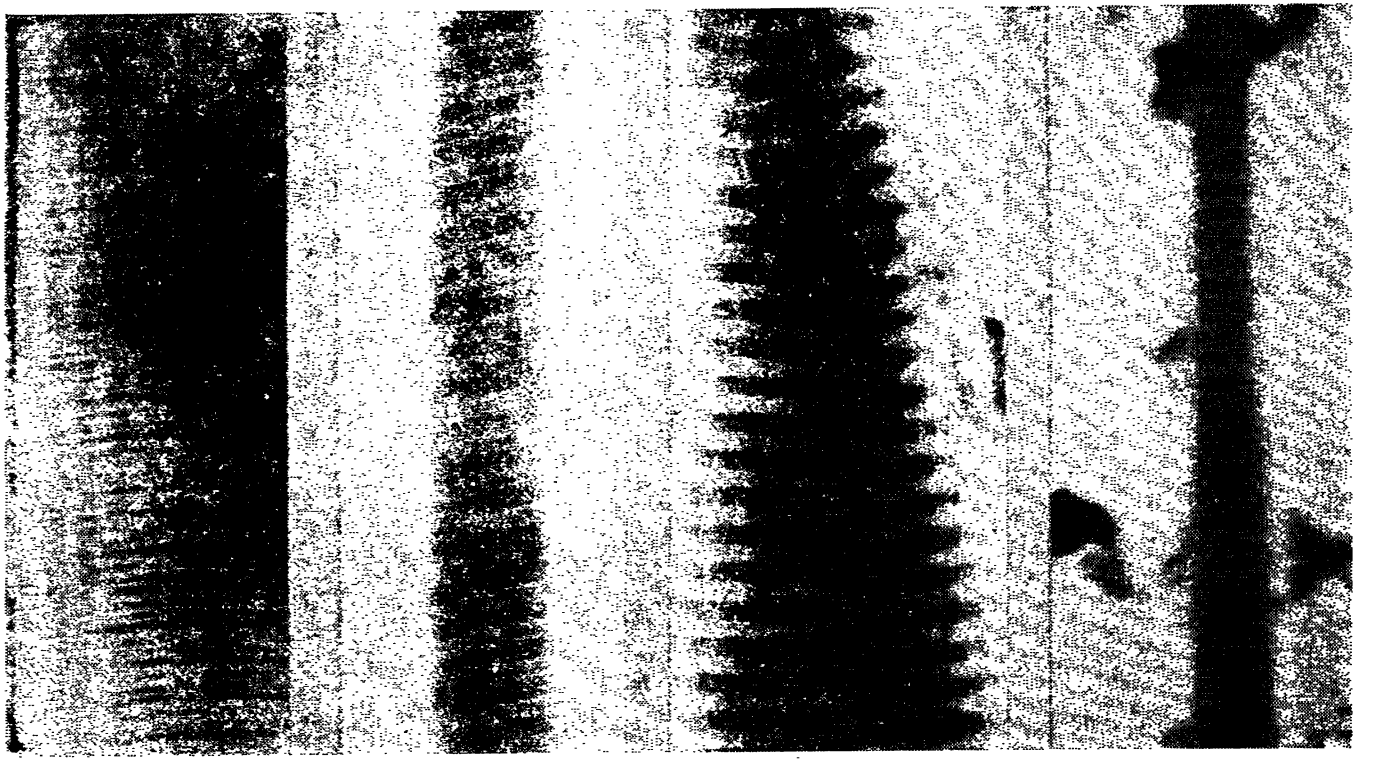

Fig. 1 *Elastoidin* (78,000 ×)

Fig. 2 *Rat snake tendon* (78,000 ×)

Fig. 3 *Dove tendon* (78,000 ×)

Fig. 4 *Horse achilles tendon* (78,000 ×)

In Fig. 1, two broad bands of about equal intensity are seen while a third band of comparatively less intensity is seen in the ligther region. Even under high magnification, further subdivisions in the bands were not seen in samples stained with uranyl acetate, or phosphotungstic acid under a wide range of conditions[14]. All the three bands are also of about the same width. The pattern, in addition, appears to be nonpolarised. Rat snake tendon fibril, on the other hand, could be resolved into a number of sub-bands by staining with uranyl acetate. The dark region contains four intrabands and at least two more are present in the light region. The differences in the intensity of the bands are also much more than in elastoidin. A similar fine structure is observed in Fig. 3, which shows a fibril from dove tendon. The stain, however, seems to be more uniformly distributed than in the case of snake tendon collagen. Fig. 4 shows fibrils from horse achilles tendon. Apart from four intrabands in the dark region, three more could be distinguished in the light region in this case.

It is well known that uranyl acetate stains by reacting preferentially with acidic groups such as the carboxyl groups of aspartic and glutamic acids[18] while the staining produced by phosphotungstic acid is due to the reaction of the stain with the basic groups like the guanidino groups of arginine[18,20]. It has been shown by superimposition of the electorn micrographs of stained collagen fibrils, that both acidic and basic amino acids occur at similar positions along the length of the collagen fibril. From the present study, it is seen that there are variations in the staining pattern of collagen fibrils from different sources. This means that there is a difference in the concentration and/or distribution of the amino acids along the polypeptide chains. The three banded periodicity in elastoidin with three broad sub-bands, suggests an altogether different type of amino acid sequence in contrast to other collagens. Apart from a localised accumulation of polar amino acids around three regions, the molecules themselves appear to be aligned laterally in an antiparallel manner[14]. On the other hand, the high intensity may also be due to high material density in these regions as a result of the presence of a large amount of extraneous materials like the non-collagenous, carbohydrate components. It may be mentioned here, that elastoidin is associated with large amounts of these substances[21,22].

Comparing the other three collagen fibrils, six, six and seven intrabands are observed in rat snake, dove and horse tendon collagens respectively. It is known that variations in the intensity could be brought about by a variety of experimental factors such as the time of staining, *p*H of the stain used and the condition of the collagen fibrils at the time of staining. These factors were kept in mind while carrying out the experiments. All the staining was

done using the same solution under identical or near equal conditions. The collagen fibrils were obtained from fibres purified by the same method. That, in spite of these precautions, there are variations in the number and the intensity of the intrabands, shows that these differences must be real. This difference in intensity shows that there had been differences in the quantitative take up of these stains by the different collagens studied.

The amino acid composition differs amongst the samples of collagen obtained from different species and, sometimes, amongst different tissues of a single animal[23]. The experimental error[23] in determining the amino acid composition due to hydrolytic losses, incomplete hydrolysis and oxidative loss is minimised by the introduction of refined techniques like column chromatography and the use of gelatins instead of parent proteins and thus fairly reproducible results are obtained. Much of the error due to the presence of nonprotein substances may be removed by expressing the composition in terms of residues per 1000 total residues. Even after applying these precautions, it is found that there are variations in the composition of different collagens[24]. The large variations in composition, therefore, could not be attributed entirely to errors of analysis and protein impurities. Though the elaborate pattern of collagen structure is known definitely from X-ray diffraction and analytical evidence, the excat sequence of the residues is not completely fixed[25].

Considerable replacement of certain amino acid residues by others thus appears possible. Such differences seem likely to be responsible for the intensity variations in the electron micrographs although they may or may not contribute to the differences in the stability and related properties of collagenous systems.

The state ot the polar amino acids may also play a part in determining the staining characteristics. Thus, the ratio of the amide content to the total acidic amino acids may affect the staining. The ratio of lysine to hydroxylysine which varies considerably between sources will also be a factor especially when samples stained with anionic stains are compared.

Another important factor appears to be the presence of bound non- collagenous impurities. These can influence the band pattern in two ways. Firstly, they may block the stain from reacting with the amino acids because of stereochemical considerations, or, they may themselves react with these amino acids and thus make them nonreactive towards the stain. Secondly, they may also influence the contrast by their mere presence at restricted places, scattering more electorns due to higher overall material density. The fact

that reconstituted collagens, with less associated impurities reveal a more detailed fine structure than their corresponding native collagens, strongly, suggests such a possibility. A study on the electron microscopy and the chemical composition of different collagens purified by Nishihara enzyme[26] and also of reconstituted collagens to explore this possibility is in progress.

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Surface Structure of Collagen Fibres as Revealed by Electron Microscopy

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The results of an electron microscope study of the replicas of raw and modified collagen fibres are reported in this paper. The surface corrugations on the fibres, observed using this technique, enable us to explain the directional friction effect (D.F.E.) of collagen fibres. Several variations from the normal structure were also observed and these are presented using electron micrographs of the replicas. Some modifications in the usual method which improved the technique for application to collagen are described.

There are certain physical properties like friction which are mainly dependent on the structure of the surfaces. When the frictional properties of collagen fibres from a variety of sources were investigated, it was discovered that they exhibit directional friction effect (D.F.E.) *i.e.*, the coefficient of static friction in one direction is different from that in the opposite direction. Various factors like source[1], age of the animal from which the samples are obtained[2], tanning treatments to which they are subjected[3] and the dosages of ionising radiations to which they are exposed[4] are found to have a pronounced effect on the structure of the surface and hence on the friction. Thus, to explain the occurrence of this phenomenon, an electron microscope study of the surfaces of collagen fibres is useful. Since the surface structure of collagen fibres does not appear to have been studied systematically so far, an attempt was made by us in this direction. Experiments designed to standardise replication techniques for the study of collagen surfaces not only yielded valuable information to explain the presence or otherwise of the D.F.E., but also some more interesting facts regarding the morphological structure of collagen fibres as such. The results of electron microscopical observations of the replicas of collagen fibres are presented in this paper.

For fibre surfaces in general, a fair amount of single stage and two stage techniques have been described in literature. Bearnes *et al*[5] have described a method of preparing a two stage replica of the surfaces of organic materials and Ramanathan *et al*[6] have described a low molecular weight replica method for the study of wool fibres. Bradley[7] has described a method of preparing a carbon replica. These methods have been widely used for the study of surfaces of organic and other fibrous materials.

As far as collagen is concerned, the reported work is scanty. Gross *et al*[8] studied the surfaces of distintegrated fibrils from human abdomen by a single stage shadowed method. Again a similar method was used by Reed *et al*[9] to study eucollagen.

In these methods, the surfaces of *disintegrated fibrils* were studied and as such no additional information could be obtained by such studies other than what can be obtained utilising shadowing techniques. Replication of the fibres as such is helpful in getting additional information regarding the structure of the fibre or fibril bundle. In our attempt to standardise a suitable technique for the study of collagen fibres, methods which were essentially similar to that of Barnes *et al*[5] were resorted to. The results of our experiments are elaborated below.

Materials and Methods

Collagen samples, except the kangaroo tail tendon which was obtained in a purified form from the Division of Wildlife Research, Australia, were collected from freshly sacrificed animals and purified following the method of Bergmann and Stein[10].

A polystyrene film was prepared by drying a pool of 5% polystyrene solution in benzene over a clean microslide. The intermediate replica was prepared by sandwiching the dry collagen fibre between a microslide and the slide containing the film and heating the assembly to 95°C for 2 hr in an air oven. The fibre was removed after the assembly was allowed to cool down for half an hour.

The polystyrene impression was then covered *in vacuo* by a silicon monoxide film, about 400 Å, thick. The polystyrene-silicon monoxide combination was examined in an optical microscope. The portions containing interesting details were cut out using a sharp razor and mounted on grids. Polystyrene was then dissolved away by benzene vapour in a reflex boiler. The replicas were examined in a Siemens Elmiskop I.

This method yielded fairly interesting results. But a few of the fibrils were, however, left over in many of the replicas and these interfered with the total field under view. To eliminate the interfering fibrils, a slightly modified method had to be followed. The polystyrene film carrying the collagen fibres partly embedded in it was dipped in clean distilled water heated to 60-65°C. The fibres shrank and were washed away. Silicon monoxide was evaporated on to these films after drying and desiccation and final replicas were prepared from the second cast.

Replicas of tanned collagen fibres and fibres exposed to ultraviolet and gamma radiations were also prepared to see the changes brought about by these treatments.

Results and Discussion

Comparing the electron micrographs of stained and shadowed collagen fibres subjected to disintegration with the replica of the fibre surface, the appearance of the fibre as a whole is not obtained in the former. In the replica presented in Fig. 1, on the other hand, we could see the fibre surface wherein several fibrils have aggregated to form a fibre. The periodicity of the major cross-striations which could be seen clearly is the same as the periodicity in the shadowed samples. From Fig. 1 it is clear that the fibrils need not always aggregate regularly even in a fibre bundle from tail tendon though the chances of such regular aggregation are greater. Incidentally a few actual fibrils could also be seen in places.

Fig. 2 explains the very process of replication method. A single fibril, after making an impression in polystyrene, has moved away. During silicon monoxide evaporation, the impression got replicated while the actual fibril got shadowed with silicon monoxide. In the final replica, the replica and the actual fibril could be seen in juxtaposition with each other. The major periodicity and the saw-toothed edges in the replica of the fibril could be observed.

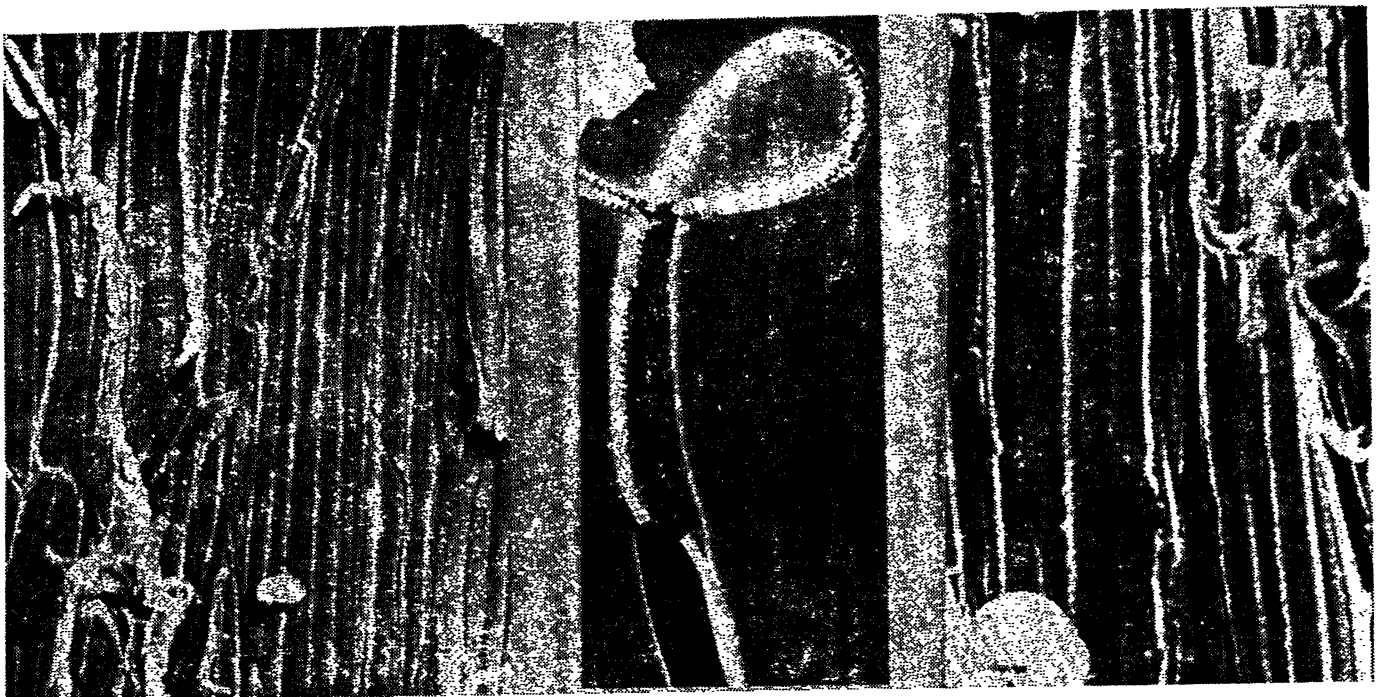

Fig. 1 *SiO replica of RTT Collagen fibre* (21,000 ×)

Fig. 2 *Fibril and replica of RTT collagen* (12,500 ×)

Fig. 3 *SiO replica of Cow Flexor Tendon* (25,000 ×)

Almost all the replicas of collagen fibres from different sources indicated in general a bundle of fibrils with major cross-striations prominently seen. Fig. 3 is an example of such normal replicas. Such electron micrographs of the replicas of collagen fibres were used to measure the periodicity of the major cross-striation using a filar micrometer and the results so obtained are tabulated in Table 1. It is interesting to note that the periodicity of the major cross-striations obtained in replicas is the same as the periodicity measured using normal shadowed fibrils[11].

TABLE 1

Periodicity of collagen fibrils in replica

Sample	Periodicity (Å)	
	Mean	Standard deviation
K.T.T.	703	38
Bull Tail Tendon	641	30
Buffalo Tail Tendon	752	32
Fox Tail Tendon	900	35
Bandicoot Tail Tendon	683	14
Sheep Tail Tendon	688	28
Goat Tail Tendon	673	19
Rat Snake Tendon	673	67
Cow Flexor Tendon	675	22
Rat tail Tendon		
1 month old	643	40
3 months old	713	34
6 months old	672	37
1 year old	657	50
2 years old	660	52

In a few of the above electron micrographs, a tendency on the part of the fibril edges to exhibit saw-toothed corrugations could be seen. Such an effect is more marked in Fig. 4 which explains the presence of D.F.E. in collagen samples.

Fig. 4 *SiO replica of Bull Tail Tendon (saw teeth)* (75,000 ×)

Borasky *et al*[13] have pointed out certain peculiar features in shadowed and stained collagen fibrils like twisting, distortion etc. Such features were also encountered in SiO replicas. In Fig. 5 four fibrils could be seen twisting about each other and in Fig. 6 where two fibrils could be seen, one is found to have distorted. This sort of distortion may either be due to the technique followed or an inherent defect on the fibrils.

Fig. 5 *SiO replica of Bull Tail Tendon (twisting)* (35,000 ×)

Fig. 6 *SiO replica of Rat Snake Tendon (distortion)* (35,000 ×)

Fig. 7 shows the replica of KTT fibres prepared after removing the fibres from the intermediate by shrinkage. This picture is seen to be relatively free of interfering fibrils.

Tanned collagen fibrils were found to be mostly coated with tanstuff as reported earlier[13,14]. A representative picture of tanned RTT is presented in Fig. 8. While the figure contains the impression of a few fibrils bunched together, the complete absence of striations could be seen. These confirm our observations on friction[3].

Ultraviolet and gamma radiations are known to destroy the structure of collagen fibrils as observed electronmicroscopically by Ramanathan *et al*[15,16]. Replication studies confirm such a view as could be seen in Fig. 9 where the surface of a collagen fibre degenerated by such treatment could be seen. As both the treatments produce more or less similar effect on the surfaces, only a single example is given.

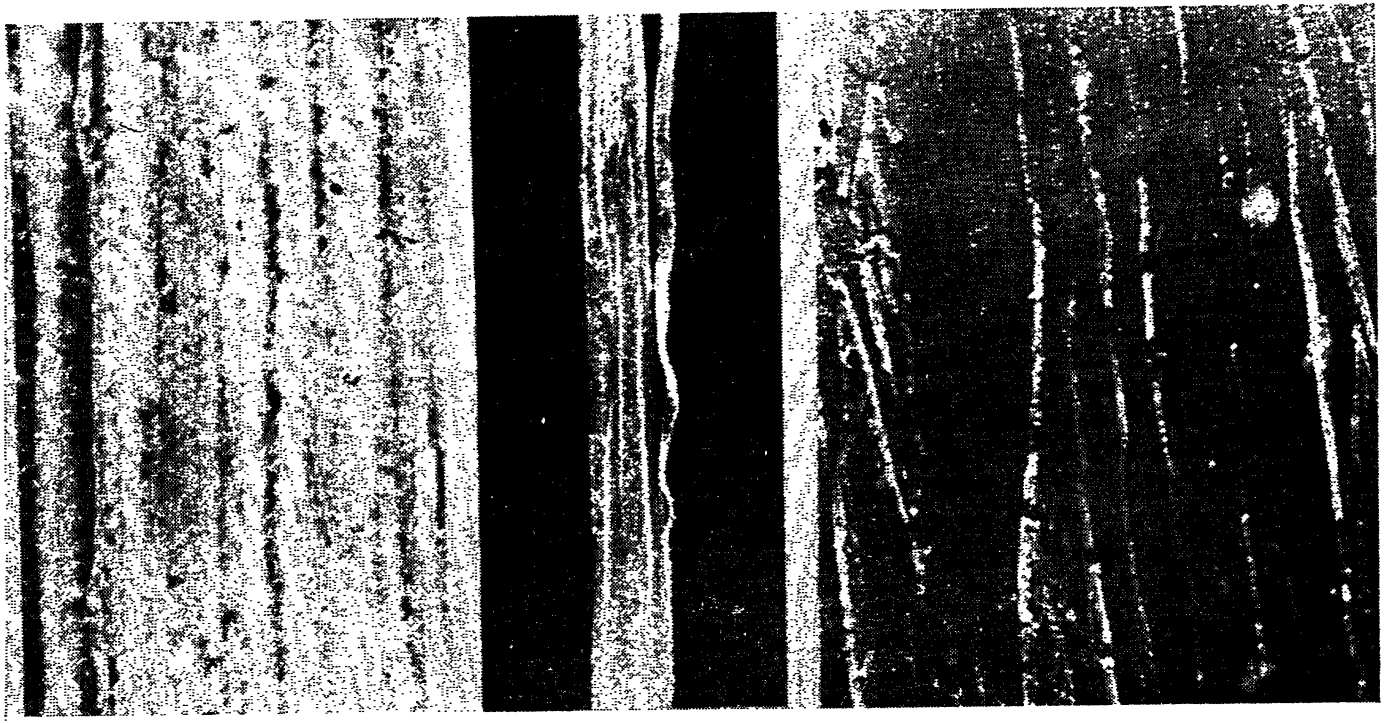

Fig. 7 *SiO replica of K.T.T. fibres prepared by shrinking the fibre for the removal of the same* (12,000 ×)

Fig. 8 *SiO replica of tanned fibre* (12,000 ×)

Fig. 9 *SiO replica of collagen surface degenerated by exposure to UV* (40,000 ×)

Acknowledgements

The authors are thankful to Dr. Y. Nayudamma, Director, Central Leather Research Institute for his kind permission to publish this paper. One of the authors (D.P.G.) is thankful to the Council of Scientific & Industrial Research (India) for the award of a research fellowship during the tenure of which this work was done.

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DISCUSSION

SESSION III

G. N. Ramachandran (*University of Madras*) : Dr. Verzar has mentioned that hydroxyproline is used as a measure of collagen. This means that in all the specimens, you have studied, the hydroxyproline content is constant. Is this true ? Amino acid content does not vary with age. On the other hand, in a paper in the London symposium at King's College in 1952 or 1953, it was indicated that in very young rats, from foetal stage upto 15 days or 1 month, the hydroxyproline increases with age.

F. Verzar (*Institute of Gerontology, Basel*) : It is absolutely like that. The collagen is just built up and, after 2 months, the rat is quasi-ready. The increase is only during this time and then hydroxyproline is constant for each collagen, say 7% for cold blooded animals and 13% for mammal.

G. N. Ramachandran : I think it is not quite correct to say that collagen is being built up, because, if I remember right, the proline content does not increase. The proline content is almost constant. It is not the amount of collagen that increases but the collagen composition changes. I think some of you have seen a recent paper by Prockop and his collaborators where he states about the hydroxylation of collagen. This is very important as collagen is characterised by hydroxyproline. I would like to know what is the role of hydroxyproline in collagen. At least I, personally, do not know what is the purpose or role of hydroxyproline in collagen. On the other hand, in early states, until the animal becomes mature and becomes just sufficiently well grown, proline gets hydroxylated. Hydroxyproline does not get incorporated into collagen if the animal is fed with hydroxyproline. It gets converted into proline and proline gets incorporated into the system and then only hydroxyproline is formed. There is an early stage, well before maturity, where there is a change in collagen (formative stages). Prof. Verzar mentioned about the old age changes in tension, which he attributes to the production of crosslinks. I would like to invite suggestions as to the type of crosslinks that are formed. Prof. Verzar has indicated two types of collagen, (1) which is produced by the addition of linkage of sugar residues as the German group think and (2) in which aldehydic and ester linkages are formed as Gallup and his group think. Are there any comments from the chemical point of view ?

Y. Nayudamma (*Central Leather Research Institute, Madras*): Has Prof. Verzar looked into the aspects, in relation to tension properties, of skins diseased or rheumatic pains etc. which may probably give an indication. We have shown that skin from the same person from belly, backbone etc. is, in some respects, different from the skin at elbow which requires a lot of flexural endurance. Is there any change in the isometric tension in such cases ? Have you looked at it from the physical properties point of view, the use of skin by the body and in relation to it as we age along ?

F. Verzar : Adoption of new collagen in the body is possible during the whole life. Collagen itself is a standard macromolecule, but not the fibroblasts which produce collagen. Fibroblasts always can produce new collagen. To the question, " what is the stimulus for collagen adoption for fibroblasts " which may just act in the way stated in the question, the

answer is—"*tension*". We have two experiments which show that the rat has a two corned uterus ; if, in one form, you put in a foreign body which stretches that, immediate collagen production from fibroblast starts and this collagen is young collagen. Similarly a foreign body under the back skin of a rat induces production of young collagen to counteract the stress. Therefore, in these cases, collagen is produced in the body. If you measure the percentage of soluble labile collagen in the abdominal skin in the very young rat, it equals that in the back skin. As the animal grows and abdominal organs grow and increase the volume of the belly, the abdominal skin has to follow by tension and new collagen is produced through fibroblasts. Hence there is collagen production in the body as needed.

K. J. Kedlaya (*Central Leather Research Institute, Madras*) : Dr. Verzar observed a difference (with respect to tension) in behaviour of collagen aged slowly and aged in an accelerated manner by exposing collagen to a relatively high temperature. I am of the opinion that this is due to accelerated surface drying similar to case hardening in the case of leather. Quick surface drying does not allow the interior of the fibre to dry completely ; in the former instance, drying is slow.

N. Ramanathan (*Central Leather Research Institute, Madras*) : We have measured the physical properties of collagen fibres from tail tendons of rats of various ages and we found that at about 6 months age drastic changes took place in the physical properties, tensile strength, refractive index etc. This could be explained on the basis of Dr. Verzar's saying that 5 months is the age of maturity in rats. The causes for this may be due to the components becoming more compact. We have found differences in the physical properties of collagen fibres from different sources and the variation of isometric tension with source described by Prof. Verzar is in accord with our findings. If the changes produced by age on collagen fibres are the same *in vivo* and *in vitro*, do the collagen fibres once produced have no relation with the body processses?

F. Verzar : The changes *in vitro* of collagen are relatively very quick (days, weeks or a few months). In the living animal, they are much slower (years). If it is a similar molecular change, then, in the body, something is active antagonistically against the age changes *in vivo* in the whole body. Of course, if this something is not acting, ageing may become quicker.

Besides this, I think that ageing *in vitro* has a different mechanism. The relation of swelling and water content to denaturation tension is different in the living animal.

The results on collagen may be similar, but the mechanisms are different. This needs further study.

B. K. Patnaik (*Regional College of Education, Bhuvaneswar*) : Mr. Kedlaya, do you propose to study calcium as a crosslinking agent in aging as calcium is bivalent ? Shelly of Canada reports that one of the causes of ageing may be accumulation of calcium in the body.

K.J. Kedlaya : As there is a general lowering of the shrinkage temperature when collagen is treated with calcium hydroxide during the liming treatment, we are led to think that such changes may not take place. But, from this, one cannot say what is taking place in the body. Further work on the basis of Shelly's findings has to be carried out.

P.S. Venkatachalam (*Central Leather Research Institute, Madras*): Is there any proposal to work on collagen from long lived animals, for instance, turtle, which is reported to live hundreds of years ?

F. Verzar: We had well-kept frogs for 17 years and the tension behavior of frog tendon was similar to that of rat tail tendon. Bullier has done some work on snakes, the scales giving an idea of the age. He found an increase in the tension in cold blooded snakes.

D. Ramaswamy (*Central Leather Research Institute, Madras*): Ageing may be a co-operative effort and we cannot individualise that it is due to one agent or another. For example Nayudamma, Jayaraman and Krishnan studied aldehydes in the presence of mineral salts, and found very high fixation to collagen though the salts were in catalytic amounts.

G.N. Ramachandran: Such an observation is interesting even in biological systems. Questions arise as to whether collagen, in non-biological systems, will behave exactly similar to ageing in biological systems and whether ageing is a question of mere age or due to biological effects. Also, as Dr. Nayudamma mentioned, calcium, along with aldehydes and other reagents, may be responsible for hardening of the tissues with age. As far as I know the collagen in bone is very similar to the collagen in the skin and tendon. Calcium goes into close combination with bone. Why does bone get calcified as contrasted to skin or tendon? What is the mechanism that prevents the calcification of the skin or tendon? Calcification may be responsible for ageing.

B.K. Patnaik: Mr. Hanumantha Rao found out a decreased level of collagen and RNA in rats fed with low protein diet or protein free diet. Is there some relationship between dietary protein and synthesis of messenger RNA (m-RNA) responsible for the production of collagen?

V. Hanumantha Rao: (*Central Leather Research Institute, Madras*): In the present investigation, only the total RNA has been estimated. It has not been possible to fractionate and estimate separately m-RNA or s-RNA which will require special techniques and equipment, for which facilities are not available in our laboratory. In general, it is known that m-RNA contains the information for protein synthesis. However, in the absence of specific data on collagen synthesis and m-RNA content, it is difficult to say if there is any direct relationship between m-RNA and collagen synthesis due to low protein or protein-free diet.

G.N. Ramachandran: When you have lesser protein, the metabolism in all systems, in all respects, goes down. I am sure, if you would have used myosin, the same results would have come. You are not able to specifically say which component in the protein intake is responsible for the collagen being lesser. It is very clear that the greater the amount of protein, the better is the metabolism to a certain extent, beyond which increases in protein does not have any effect. Collagen is one of the products of metabolism in the system. Therefore, everything acts together and, apart from that, have you not been able to establish this with respect to certain amino acids? For instance, it is more interesting to see how the amount of collagen production is correlated to the amount of proline content and see whether proline, in particular, has that effect and if glycine has that effect. When you give the total protein, one loses the track. You have analysed the glycine content and found some correlation for that. This is the sort of thing that gives more information as to the precise mechanism of collagen formation rather than a general study of the relationship between protein intake and collagen formation.

S. M. Bose (*Central Leather Research Institute, Madras*): This is a part of nutrition study and the effect of the basic protein in the animal body. We have to take care that, in the diet, there should be all the essential amino acids. If we deprive the diet of any of the

essential amino acids. our experiments would fail. Even if we give extra amounts of proline, we have to give a complete protein in the diet. So we have given casein from 0% to 55%. In the standard concentration (20%), if you give extra amount of proline, I do not think it would serve any purpose since the body has a limit for acceptance or incorporation of proline or conversion of proline into hydroxyproline and then incorporation in the peptide linkage. Nutritional experiments cannot be carried out by excluding essential amino acids and selecting particular amino acids like proline or glycine. It is an experiment planned in such a way that we got six sets of results from the total content of collagen, procollagen or neutral salt soluble collagen, radioactivity of gelatin and the free glycine and on the other side, we take metabolism, urinary hydroxyproline and the plasma hydroxyproline. If you kindly view the six sets of results and if you study critically, you will find this interesting observation that while we deprive the animal of the standard level of 20% protein and allowed say 6%, we get certain systematic change in the constitutents whereas if you increase the level above 20%, there is absolutely no difference. In the case of DNA, there is no change irrespective of the level of protein or protein free diet while there is a change in all the other components studied.

G.N. Ramachandran : With ultraviolet radiation, Messrs. Guha and Saha found the water soluble fraction to be increased. Apparently, there is something like an increase of the soluble part, a decrease of shrinkage temperature and general denaturation of the specimen when irradiated. If this is the general thing to be expected, I thought that, in a way, it is reasonable because, in irradiation, chemical action takes place. This effect is much more when gamma radiation is used instead of ultra violet. UV has the effect of chemically affecting certain bonds. I do not completely agree with the statement that it may be breaking hydrogen bonds because breaking of hydrogen bonds cannot lead so easily to production of soluble collagen. My feeling is that there is something in the nature of the bonds that keeps the different parts of the collagen molecule together, as it happens in the case of the tropocollagen molecule, the bond that connects the alpha and beta chains. It is something of that nature that has broken. Therefore, the collagen molecule just gets out of the whole fibre and gets into solution. There is a feeling those bonds are covalent in nature, but the exact nature of these bonds is not known. In thermal denaturation, the amount of soluble collagen is increased. Generally, the effect of irradiation seems to be similar to the effects produced by denaturation.

V. Mohanaradhakrishnan (*Central Leather Research Institute, Madras*) : Prof. Ramachandran has said that with increase in irradiation a larger number of crosslinkages might break and soluble collagen may come out. It has been shown by Bowes, Cassel and others, by molecular weight determination and by chemical methods, that it is the peptide bond of the molecule that is broken in irradiation. The whole molecule does not come out.

N. Ramanathan : Is it necessary to assume also the breakage of bonds other than H —bonds to explain increased uptake of tanning materials ?

N.N. Guha (*Saha Institute of Nuclear Physics, Calcutta*) : No. Vegetable tannins mainly fix on the peptide bonds which are not internally compensated by hydrogen bonds. Since hydrogen bonds are weak, these are expected to be broken by UV irradiation easily making some peptide bonds free for further uptake of tanning material, specially those adjoining the residues of phenylalanine, tyrosine and tryptophan. It is known that UV radiation is mainly absorbed by aromatic amino acid residues. The other groups which might be involved in the fixing process contribute to the same extent before and after irradiation.

G.N. Ramachandran : Messrs. Goverdhan Rao and Ramanathan, would you explain the pattern seen in the electron microscope to justify the directional friction effect or D.F.E.?

N. Ramanathan : Inclined corrugations (similar to saw-teeth) were observed.

G.N. Ramachandran : Has any attempt been made to correlate the direction with the corrugations seen in the electron microscope?

D.P. Goverdhan Rao (*Central Leather Research Institute, Madras*) *:* Due to experimental difficulties, it was not possible to correlate the direction of the fibre with the structure obtained electronmicroscopically.

G.N. Ramachandran : Have you never found the corrugations pointing in opposite directions in the same micrographs ? It would have been better if the fibres were first studied under an optical microscope and then the results were correlated with the electron micrographs.

D.P. Goverdhan Rao : No. As studies at optical level had already been carried out by Prof. Verzar we thought it would be a repetition. Further, during the measurement of friction, the fibres were observed at low magnifications and surface non-uniformities were seen.

F. Verzar : I have found cable-like structures, similar to striations in optical pictures. I think these are, to a great extent, responsible for the D.F.E.

Technical Session IV

Investigations on the Therapeutic Efficacy of Indigenous Drugs Against Skin Affections of Livestock

V. MAHADEVAN AND R. K. GUPTA

Indian Veterinary Research Institute, Izatnagar, U.P.

Systematic chemical and pharmacological studies have been undertaken to investigate the usefulness of indigenous drugs against skin affections of livestock. A number of indigenous medicinal plants have been investigated to evaluate their therapeutic efficacy against two of the very serious skin ailments of great economic importance, namely mange and ringworm. The results of investigations on two indigenous drugs, *Cedrus deodara* and *Cassia tora*, which have been found to possess remarkable curative action against mange and ringworm respectively are discussed with illustrations.

There are a number of skin affections encountered amongst livestockl which cause appreciable damage to hides and skins besides having a harmfu, effect on the development of young animals and lower the productivity of adults. These affect a very large number of animals every year and the blemishes caused by them on hides and skins greatly reduce their quality and thus fetch comparatively a very low price in the international market. In spite of India being a leading exporter of hides and skins, it lags behind in the quality of her raw stock.

A wide variety of synthetic insecticides and proprietory medicines have been recommended from time to time to combat these ailments, but most of them suffer from certain drawbacks. Some of these are not easily available to an average livestock owner or are fairly costly, while a few indirectly

produce acute toxicity to the host or sometimes the parasite itself develops immunity towards certain drugs. It has been observed in the case of few novel insecticides that these are absorbed by the body tissues of the treated animals and later on their toxic residues are eliminated in the milk and milk products thus constituting a great public health hazard. These observations necessitated to investigate the usefulness of indigenous drugs for the treatment of certain skin ailments of livestock. Therefore, a detailed investigation has been carried out on a number of indigenous medicinal plants. The present communication deals with the chemical and pharmacological studies on two indigenous, medicinal plants, drugs, *Cedrus deodara* and *Cassia tora*, which have been found to be quite effective against two of the common skin infestations, mange and ringworm respectively, which are known to cause considerable damage to hides and skins.

C. deodara is an evergreen tree growing plentifully in the north western Himalayas at an altitutde of 4000-12000 feet. The tree yields one of the finest timbers and its various parts are attributed to their medicinal uses. The wood is reported to possess diaphoretic, diuretic, and carminative properties besides being useful in skin diseases[1,2]. The wood oil popularly known as *deodara oil* has been chemically examined by earlier workers,[3,4] but no systematic work has so far been done to substantiate its medicinal properties. A detailed study has been undertaken to evaluate the therapeutic efficacy of *deodara oil* against mange (sarcoptic, psoroptic and demodectic) in livestock.

Materials and Methods

The *C. deodara* wood powder was exhaustively extracted with light petroleum (b.p. 40-60°) by cold percolation. The combined petrol extract on removal of the solvent by distillation yielded a brown coloured viscous oil (yield 2.4-2.8%) which was subjected to steam distillation. The steam volatile fraction was taken in ether and the ether layer was extracted with 5% aqueous sodium hydroxide solution to remove fatty and resinous impurities. The ethereal layer was then washed with distilled water and dried over anhydrous sodium sulphate. It was then distilled to give a pale yellow oil (1.4-1.6) having a characteristic balsamic odour. The oil thus obtained was employed in all the experimental trials and, henceforth it will be referred to as processed *deodara oil*. The physical and chemical characteristics of the

processed *deodara oil* in the present study and those reported by Simonsen and Rau[3], are given below:

	Processed deodara oil (1965)	Values reported by Simonsen and Rau[3]
1. Colour	Pale yellow	Dark brown
2. Density	0.9309	0.9592
3. Refractive index	1.5149	1.5203
4. Optical rotation	+ 76°	+ 68°
5. Acid value	0.2	1.5
6. Saponification value	6.55	18.77
7. Saponification value after acetylation	42.3	22.32

The difference in the values of the above two samples appears probably due to the alkali treatment of the processed oil.

Results and Discussion

SARCOPTIC MANGE : Sarcoptic mange is caused by the invasion of burrowing *Sarcoptes scabiei* mites (Fig. 1a). The disease is diagnosed by the

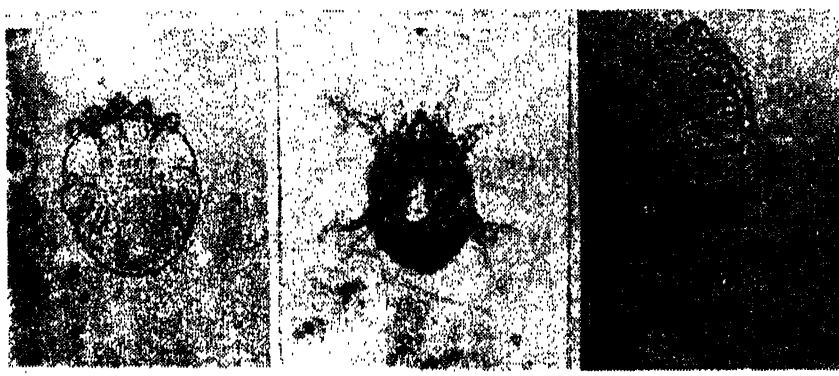

FIG. 1 Causative Mange mites

a. *Sarcoptes* sp. b. *Psoroptes* sp. c. *Demodex* sp.

presence of mites and their eggs in the skin scrapings and is clinically characterized by severe itching, wrinkled skin devoid of hair and erythematous patches. Folicular papules are noticed on thighs, neck, scapular regions, shoulders and forehead and in certain cases even upto hoof and tail end. The mites

penetrate deep into the skin causing intense irritation and inflammation followed by destruction of sweat glands and rupture of blood capillaries. The serous exudate on drying forms crusts on the body. Severe infestations greatly damage hides and skins and result in a marked drop in lactation, loss of condition and finally death of the animal.

Treatment trials were conducted on buffalo calves suffering from sarcoptic mange (Fig.2). A ten precent solution of the processed *deodara oil*, in castor

FIG. 2 Buffalo calf suffering from *Sarcoptic* mange
(Top) Before treatment (Bottom) After treatment

oil or petroleum jelly was externally applied on the affected parts of the animal with a soft brush once on alternate days. The progress of the treatment was determined by periodic examination of skin scrapings for the presence of mites. The intensity of mites was gradually lowered after second application of the drug and no mite was found in the scrapings after the fourth application. However, two more applications of the drug were given to ensure complete cure. The scratching amongst the treated calves were considerably reduced after the application of the drug. During the experimental trial a few animals were kept as controls and no treatment was given to them. These animals retained the same intensity of infection and in some cases the general condition further deteriorated. Subsequently the control cases were also treated with the above drug. Different concentrations of the processed *deodara oil*, were used in separate treatment trials to determine the optimum concentration to give effective cure within the shortest time. Ten percent concentration of processed *deodara oil* in castor oil base or petroleum jelly gave the best curative results. The treated animals showed significant improvement in their general condition and appeared healthy. The animals were kept under observation for a period of two months and no recurrence of the disease was noticed. The hair growth on the bald patches of the treated animals was noticed after 2-3 weeks. The above concentration of the drug was tried on several repeated attacks and it gave uniformly curative results. The drug showed no contra-indication during or after the treatment.

Histopathological examination of the biopsied portion of the skin affected with mange showed extensive hyperkeratosis of stratum corneum with fragments of pycnoctic nuclei interspersed in structureless material. However, no cross section of adult mite was seen. Granulomatous changes were noticed in the dermis as evidenced by dilatation and congestion of many newly formed capillaries accompanied by infiltration with fibroblasts, lymphocytes, plasma cells and a few neutrophils. The capillaries were seen directed at right angle near the necrotic debris.

The same area of the biopsied skin after treatment showed an uninterrupted continuity of the epidermis with all layers of cells constituting it. The dermis did not reveal numerous dilated and congested capillaries as seen in the affected skin. However, stray clusters of fibroblasts and lymphocytes were observed. Fibroblasts had laid down collagen at some places (Fig. 3 a and b). This indigenous drug not only completely cures the disease, but

also restores and regenerates the normal skin on the affected parts of the body after treatment (Fig. 3).

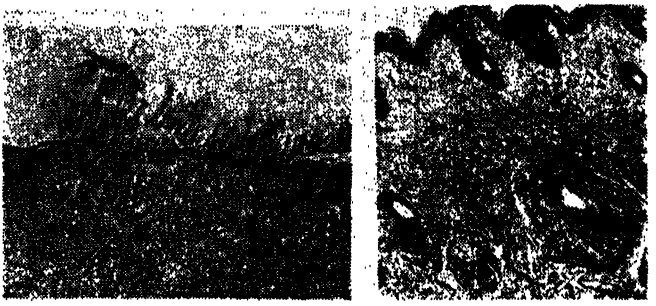

FIG. 3 Histological sections of the skin affected with *Sarcoptic* mange
a. Before treatment b. After treatment

PSOROPTIC MANGE : Scab mange is a very prevalent and highly contagious disease caused by the mites of the genus *Psoroptes* (Fig. 1b). The disease is principally encountered in sheep, cattle, goats, horses and rabbits. The mites are host specific and normally parasitise on parts of skin which are thoroughly covered with hair or wool. The mite pierces the skin, sucks the blood and sera and simultaneously secretes a toxic substance. This causes intense irritation, inflammation and scratching resulting in the destruction of sweat glands, impairment of hair follicles, exudation of serum and also bleeding in severe cases. The infected parts lose their hair and scabs appear on the skin. When a large area of the body becomes denuded of wool, the sheep perish during winter due to exposure and loss in general condition. The scab mange not only causes appreciable damage to hides and skins, but also adversely affects the wool production in sheep and hampers growth and productivity of other animals.

The treatment trials were conducted on sheep and goats sufffering from psoroptic mange. The same line of treatment was followed as in case of sarcoptic mange, but, using only half the concentration of the drug *i.e.*, five percent solution of the processed *deodara oil* in castor oil base or petroleum jelly. The drug was supplied externally on the affected parts once on alternate days for 8-12 days depending upon the intensity of infection. It was found to be highly effective against psoroptic mange without showing any untoward side effect on the skin of the host. The drug restored the skin

to normal healthy condition followed by a marked improvement in the general condition of the treated animals (Fig. 4).

FIG. 4 Sheep suffering from *Psoroptic* mange
(Top) Before treatment (Bottom) After treatment

DEMODECTIC MANGE : Demodectic mange causes enormous damage to hides and skins at times rendering cattle hides completely unfit for leather manufacture. The disease is caused by mites of the genus *Demodex* (Fig. 1 c) and is commonly seen in cattle, sheep, goat, dog and also in man. The causative organism is noticed in pustules and outside in the sub-cutaneous tissues. Clinically the disease is characterised by loss of hair, wrinkled skin, exudation of lymph, absence of itching and formation of pustules and crusts. The lesions are seen of the size of millet to walnut seeds on any part of the body.

The treatment trials were conducted on cow calves suffering from demodectic mange. A twenty percent solution of the processed *deodara oil* in castor oil or petroleum jelly was applied on the affected parts of the animals with one external application on alternate days. The drug slowly penetrated the crusts and pustules expelling them of their contents and making them dry finally. The treated animals were cured on 2-4 applications of the drug. The above concentration of the drug was found to be necessary to get efficacious results against demodectic mange and was found to have no undesirable action on the skin of the treated animals.

Investigations on Cassia tora : *C. tora* is an annual wild herb which grows profusely throughout India. It does not require any proper care for its cultivation. The seeds of the plant are described to be useful against skin diseases,[1,2] and have been chemically investigated by several workers[5-8]. The seeds of the plant were subjected to a detailed chemical and pharmacological study to assess their efficacy against ringworm in cattle.

Isolation of the active principle : The seeds are milled to a coarse powder which is then exhaustively extracted with alcohol (95%) under reflux. The solvent was distilled off and the alcoholic extractive was treated with excess of light petroleum to remove the fatty oil. The defatted residue was hydrolysed with seven percent hydrochloric acid and thoroughly extracted with ethyl acetate. The ethylacetate extract on removal of the solvent and careful crystallisation yielded a micro-crystalline yellow product, m. p. 194 - 6°. It was, analysed and characterised as chrysophanic acid, an anthraquinone derivative on the basis of its colour reactions, analytical data and preparation of acetyl and benzoyl derivatives. Chrysophanic acid appeared to be the active principle of the *C. tora* seeds and was used in all the treatment trials against ringworm in cattle.

RINGWORM: Ringworm is a fungal disease caused by the fungus of the genus *Trichophyton.* The disease is diagnosed by the microscopic examination of the skin scrapings of the affected parts. Ringworm is one of the most common diseases found among sheep, goat, and more particularly cattle. The disease is not fatal save for a few cases where the severity of the infection is immense, and it brings about a reduction in the quality of hides and skins.

Mode of treatment : The treatment trials were conducted on cow calves suffering from severe ringworm infection. One percent solution of the active principle in castor oil base or petroleum jelly was applied externally on the affected parts of the body once a day keeping a few infected animals as control in each experimental trial. The treated animals recovered

completely within eight to ten days whereas the control group either retained the same intensity of infection or deteriorated further. The recovery was confirmed by the clinical examination of the skin scrapings. Normal luxuriant growth of hair was noticed on the treated parts after two weeks (Fig. 5). The drug did not show any contra—indication.

FIG. 5 Cow calf suffering from Ring Worm
a. Before treatment b. After treatment

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A Resume of Observations on Skin Tumours in Bovines

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Neoplastic condition forms an important and primary affection of the skin and adnexa in bovines. An analysis of 622 consecutive bovine tumours (in the Department of Pathology of this College) revealed that 153 of them were located in the skin and adnexa. These in the order of frequency were ; Squamous-cell carcinoma (42), Melanoma (38), Papilloma (26), Fibroma (18), Fibrosarcoma (9), Neurofibroma (4), Leiomyoma (2), Myxoma (2), Fibrolipoma (2), Basal cell carcinoma (2), Papillary carcionoma (1), Fibropapilloma (1), Follicular lymphoma (1), Myoblastoma (1), Sabeceous adenoma (1), Angiosarcoma (1), Rhabdomyosarcoma (1), and Dermoid cyst (1).

A detailed study of the nature of diseases that affect skin has largely been neglected in animal medicine. It is not often realised that several diseases, primary and secondary, affect the integument. Both infectious and non-infectious including nutritional diseases, cause damage to the skin, thus, rendering it less valuable to the leather industry. It is common knowledge that tumorous growths, affect skin of herbivorous animals, but the excat economic loss therefrom has not been properly assessed. Besides the local damage done at the site of tumours, the malignant varieties can also cause metastatic growths and thus endanger the life of the animal.

Several authors have recorded the incidence of skin tumours in bovines. In a study made earlier from this Department, 433 skin tumours were found among the 2 003 examined during the 12 year period ending 1951[1]. Of the 1,373 bovine tumours listed 134 were from skin and related structures[2]. Head[3] encountered 96 tumours in bovines among the 854 skin tumours from domestic animals. Misdrop[4] recorded 24 tumours in this situation among the 234 tumours in cattle.

In the present investigation, among the 622 consecutive bovine tumours received in the Department of Pathology for histo-pathological diagnosis, 153 were from the skin and related structures. The incidence of the different types were : Squamous-cell Carcinoma (42), melanoma (38), papilloma (26), fibroma (18), fibrosarcoma (9), neurofibroma (4), leiomyoma (2), myxoma (2), fibrolipoma (2), basal-cell carcinoma (2), sebaceous adenoma (1), papillary carcinoma (2), fibropapilloma (1), follicular lymphoma (1), myoblastoma (1), angiosarcoma (2), rhabdomyosarcoma (1), and dermoid cyst (1).

Results

Squamous-cell Carcinoma: This is the most common tumour in this series, occurring on the skin of vulval lips (21), perineal and anal region (5), abdomen (3), neck (3), buttocks (2), shoulder (1), stifle (1), knee (1), back (1) and hoof (1). (In three instances the sites were not recorded). All were in adults, 34 in females and 6 in males (ages of 2 cases were not recorded.) It is significant that a majority of them (50% of cases) occurred on the skin or/and mucocutaneous junction of vulva. There was surface ulceration acanthosis and probably papilomatous change preceded cancer formation.

Squamous-cell carcinoma of vulval skin appears to be a definite disease entity, possibly related etiologically to solar radiation. The same type of tumour noted in adjacent areas, *viz.*, perineum and anal region might be of a similar etiology. Relationship of pigmentation of the skin of the affected area and photosensitising action of plant pigments in the causation, needs further study. In this connection, observations made by Burdin[5] in Kenya on cancer of non-pigmented vulval skin of Ayrshire cows, bear close similarities. Also pertinent in this respect are the comments of Lloyd[6] and Carne, *et al*[7] on the skin tumours in merino sheep in Australia.

Melanoma: This tumour is the next in the order of frequency. The parts of the body affected were : Limbs (below hock and knee) (15), chest wall (5), dewlap (3), abdomen (3), tail (3), neck (2), ear (2), shoulder (1), buttocks (1), flank (1), forehead (1), and thighs (1). Among the 31 animals affected — one was recorded in a bull-calf aged 10 days, two in calves about 1 year and 2 in calves aged 2 years. The remaining cases were in adult bovines above 3 years. The size of the tumour varied considerably, some being very large, causing a protuberance externally. Only two of the affected animals were of brown colour, the others being either white or grey. Thirty cases occurred in males. Since follow-up histories were not available in these cases, it is difficult to comment on the degree of malignancy of melanotic skin tumours.

However, it was quite certain that some of them were malignant as confirmed by the histological studies.

It is noteworthy that nearly half the number of cases occurred on the skin of limbs below knee and hock and that majority were in white or grey animals. In six instances the tumours occurred in young animals. The presence of melanomata in young bovines have been noted by several investigators. In the series of 96 tumours, Head[3] recorded 23 subcutaneous melanomas, and most of these were in calves. The biological aspects of these tumours have not been worked out in detail nor their origin in bovines adequately known.

Papilloma (warts) : In the present series there are 26 examples of papillomas, occurring in different parts of the body or as multiple generalised lesions on the body. Majority of the latter type occurred in young animals. These are cases of infectious papillomatosis, often termed as infectious verrucae (warts). Grossly the growths appeared as small round elevations and digitata papillae. At times they coalesced to form larger ones which have a cauliflower-like appearance.

The etiological agent of infectious papillomatosis has been established to be a virus, and the reports reveal its contagious nature. In certain herds, outbreaks, of the disease, involving several young bovines, have been recorded. Spontaneous recovery may occur in the affected animals and immunisation has been found effective. The bovine wart virus appears to be the causative agent of the transmissible fibropapillomatosis of bovine genital mucosa.

Fibroma and fibrosarcoma : In this series, 18 cases of fibromas have been recorded in the following sites on the body; neck, head, forearm, axilla, around the anus, yoke place, abdomen, shoulder and brisket. No site of prediliction has been noticed.

Fibrosarcoma occurred in 9 instances. The sites were, nape of the neck (yoke place) (3), cheek (3), croup (1) chestwall (1) and tail (1). In three cases, the growths were anaplastic.

While fibromas occurred generally in young animals, its malignant counterpart occurred in older ones. It is noteworthy that in 3 working bullocks, fibrosarcoma occurred on the yoke region, suggesting a possible etiologic relationship to yoke injury.

Neurofibroma : Neurofibromas have their origin in the connective tissue of nerve sheaths, and are occasionally seen under the bovine skin, as single or multiple growths. The growths are generally small, nodular, hard and

greyish-white. The sites of occurrence noted in this series were : neck, ear, chestwall and upper part of the metacarpal region.

Leiomyoma : This tumour is uncommon in bovines, and have been noticed in two instances: One on the yoke place in a bullock, and another on the skin of the udder in a cow.

Myxoma : This is one of the rare tumours in animals. The two instances recorded in this series occurred in adult bovines, on the buttocks and chestwall respectively.

Sebaceous adenoma and basal-cell carcinoma : While both these tumours are of frequent occurrence on the skin of the dog, they are rare in bovine species. Among the three cases recorded one was sebaceous adenoma occurring on the vulval skin in cow, and the other two were basal-cell carcinoma, of which one occurred in the same location.

Fibrolipoma and fibropapilloma : There are 3 records of the above mixed benign tumours two of the former occurred on the abdomen of a cow and on the pastern in a bullock, and one of the latter occurred on the brisket region of a cow aged 8 years.

Follicular lymphoma : While lymphoid tumours involving viceral lymph nodes are not uncommon in this country, localised lymphoid tumours involving the skin and or subcutaneous tissue, are rare. Though the disease is variously classified, often two forms are recognised; an endemic type, occurring in older bovines and a non-endemic type, existing in young animals. While the etiology of the former is believed to be a virus, the causative agent of the latter is not clear.

In the form recorded here, termed more appropriately a leukosis or lymphosarcoma, occurred in a calf aged 12 years.

Angiosarcoma : A single case of this tumour has been diagnosed in a cow on the abdomen. It is likely that this is similar of the condition recently described[8] as bovine cutaneous angiomatosis, wherein the growth started as a small lesion in the dermis with intermittant haemorrhage.

Myoblastoma : A tumour on the neck of a bullock resembling myoblastoma histologically, has been recorded in this series.

Rhabdomyosarcoma : An incidence of this rare malignant tumour has been observed on the skin of a bullock on the pastern region.

Dermoid cyst : The socalled 'dermoid cysts' of the skin are often malformations and are true neoplasms. They resemble closely the dermoids seen on the eye-lids, conjunctiva and cornea of very young calves. A dermoid cyst on the poll region was recorded.

Discussion

It is clear from this survery that a variety of tumours affect the skin and adnexa. The lesion could cause both direct and indirect damage to the skin. Direct effect of the tumorous condition would be the loss of hide in the portion affected this being great in generalised conditions, such as infectious papillomatosis and multiple neurofibromatosis. This also indirectly deteriorates the quality of the hide through cachexia from maligant tumours, of which there are many. The economic loss resulting from these conditions and consequent deterioration in quality of the hide could be considerable.

This survey reveals that among the cutaneous tumours the most frequent types are : Squamous cell carcinoma, melanoma and papilloma (warts). A suprisingly high incidence of squamous cell carcinoma of the vulval skin, perineum and adjacent skin has been noticed. On an analogy with the observations made on similar tumours elsewhere in the world, it could be suggested that the etiological factors concerned might be intense solar radiation and lack of pigmentation at the site. However, further study is needed to assess the relative role of these and other factors, if any, in the genesis of this type of tumour.

It is interesting to note that the next frequent tumour in this series namely melanoma occurred in animals which included a calf aged 10 days. The biological features and histogenesis of this tumour in young animals are poorly understood. It has been suggested that the genesis of the tumour is related to disturbances in the pigment metabolism.

Generalised papillomatosis of the skin is common in young animals and the permanent damage this may cause to the hide has already been mentioned. It is stated by one authority that a large abattoir in United States of America, recorded an incidence of 15-25% of this lesion on the hide in certain seasons of year. Since the prevalence of the disease is high in certain herds occurring occasionally as outbreaks, immunisation procedures would be worthwhile under such circumstances.

The present survey has revealed several other tumours, *viz.* fibroma, fibrosarcoma, neurofibroma, and leiomyoma, which have occurred on the neck at the yoke region. It is suggested that prolonged trauma from yoke may play a significant role in the causation of tumours at the site.

Unfortunately, excepting cutaneous papillomatosis, none of the other neoplasms of the skin, are preventable. Nevertheless, skin tumours by virtue of the obvious nature of the lesion, are detected early and could be treated before they extend further or cause metastatic growths, if they are malignant.

The study of the bilogical aspects of the skin and adnexa and the tumours arising from them, is both fascinating and challenging. There is scope for a greater consideration of these problems in any scheme or work designed to maintain the healthy state of this important structure of the body.

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The Impact of Preventive Veterinary Medicine and Animal Health on the Quality of Hides, Skins and Leather

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Hides and skins are valuable by-products of slaughter house. Persisting primitive and traditional animal husbandry practices, impede promotion of trade in quality hides, skins and leather. A realistic policy must be spelled out to harvest profitably all the livestock products. Various diseases are responsible for the all round poor performance of our livestock. A knowledge of the epidemiology of the disease in a particular area and of the local husbandry practices would be needed to institute curative and preventive measures effectively. Mal-nutrition and parasitism coexist and the disease processes are aggravated. A proper evaluation of the losses due to the above, will highlight the role of preventive veterinary medicine has in this industry, which earns a sizable foreign exchange. This stresses the need for unstinted support for research, extension, education and their effective application.

Of the sevesal by-products from a slaughter and meat packing establishment, the hides and skins form the most valuable foreign exchange earners through the leather industry. The fallen hides or skins *i.e.*, those collected at death from natural causes or following ailments are not physiologically and histologically the same as skins and hides recovered at a slaughtering establishment. They are generally poor and inferior in substance and grain when tanned.

Livestock are maintained in most countries as a source of food for man, apart from the other secondary uses they are put to. Food of animal origin, in the form of meat, milk, egg etc. is indispensable for human nutrition. The livestock deserve more consideration by our planners, administrators, veterinarians, animal husbandsmen, scientists, and agriculturists because these animals are capable of converting agricultural by-products and forage not normally consumed by man, into food useful to man. Such food of animal origin can improve the nutritional level of the people. The foreign exchange earned through the hides and skins derived from livestock can raise the national economy ; this will in its turn generate effective demand for the nutritional foods. It has been demonstrated, that the number of meat animals and the production of meat reflects the prosperity and industrial growth of a country. The essential difference between the diets in developed countries and less developed regions lies in the use of livestock products.

A problem faced in the promotion of trade in quality hides, skins and leather and of livestock health and production is the persistence of primitive and tratidional livestock husbandry systems, which frequently reflect social and religious considerations rather than the needs of nutrition and economics. It is obvious that to increase the productivity of our livestock, a disease free position must first be ensured.

A poor performance in our cattle, sheep and goats is caused largely by diseases of various kinds, and particularly parasites. A parasite like the *Haemonchus*, which affects both our cattle and sheep, can release about 10,000 eggs per day continuously through a year. The survival rate of the free living eggs is so high that an infection from this parasite can be extensive and it can cause an insidious economic loss (not apparently noticed) to livestock. The damage caused can be more in sheep than in cattle, and indirectly to the skins and hides. In the case of *Anchylostomes*, a single worm can draw away, 0.5 *ml* to 1 *ml* blood per day. The damage caused by the other parasites in our sheep and cattle can be similarly estimated and it would be possible to make an assessment of the tons of blood loss in our livestock and the equivalent of feeds and forage needed to make good the loss and assess, in terms of rupees, the crores annually lost to the farmer in our country due to such infections, resulting from poor managerial skills, and husbandry practices. In effect such nonapparent loss may far exceed the loss caused by the apparently fatal specific diseases like Foot and Mouth, Black-quarter, Rinderpest, Haemorrhagic septicaemia and Anthrax. The effect of such endo- and ecto-parasitic infections on the hides and skins, in terms of money, poor quality and dampening the trade can be envisaged. Veterinary medical problems are enormous, extensive and complex in our country and hard to

be comprehended by the lay mind. The control and elimination of these conditions can be made through patient research and time-consuming implementation of newer methods in the field through extension-education of our stock owners.

The understanding of the mechanism involved in the transfer of an infective organism or parasite from one host to another is of considerable importance. If the sequence of events that precede infection is known, it may be possible to interrupt it at a vulnerable point, for control. Disease is the result of interaction between the host and parasite populations. In this sense it transcends, bacteriology, virology or parasitology of the individual and assumes significance as a problem of interspecies competition. The real need is for an epidemiological study with the objective of applying preventive veterinary medicine to reduce the economic loss due to disease and parasitic infection, in our livestock. In the application of preventive veterinary medicine, it must be borne in mind that parasites cause harmful effects on livestock efficiency ; that parasitic disease will depend on numbers, or in other words the dynamics of the parasite population ; that the parasitic disease affects the flock or herd and if one individual in the flock or herd is affected all of them may need to be treated to reduce further infection and raise the population resistance and efficiency. From the epidemiological point it would seem necessary to think of the disease and the predisposing conditions, along with the cumulative effects of parasitic disease and the effects of poor nutrition among our stocks.

While the outbreaks of specific bacterial and viral diseases are demonstrable through mortality, the loss caused by continuous but non-apparent parasitic infections is immense. Many of these animals are infected throughout life and the young respond more severely to the infection than the old. These ecto-and endo-parasitic infections frequently aggravate the conditions of malnutrition, and limit the efficiency yield of meat, milk and skins and hides. Further, the expenses towards control measures are considerable, because therepeutic treatment with an anthelmintic and other applications are frequently not effective in the face of poor husbandry practices. Without the knowledge of epidemiology of the diseases for the respective areas, the drugs and anthelmintics may be used indiscriminately, and not at the proper time. The application of control measures will imply a knowledge of the methods of animal husbandry, practiced in the area concerned and the field veterinarian can play an important role because manifestation may vary from place to place and even season to season. This situation calls for local research to deal with the problem in an informed way.

So poor husbandry practices, disease level, and parasitism prevent the maximum production of our livestock. Under these conditions the present level of poor production is accepted by our farmers as their best. Even the poor levels can be further lowered as disease and parasitic burden are added to the existing limiting factors of poor nutrition and husbandry. Field studies made in recent years in some sheep producing countries where parasitism has been suppressed, showed a significant increase in average body weight gains over infected controls. Similar results, were obtained by the Animal Husbandry Department in certain flocks of sheep in field studies in Vijayawada and Ramnad Districts in 1950-51. Field studies made on the effects of parasitism showed significant reduction in mean live weight of sheep ; there was a significant reduction in lean meat and fat in the dressed carcasses which also gave a relatively higher shrinkage of meat on storage. A fair assessment of what parasitic and other diseases in our sheep and cattle cost us and of the loss to the hides and skin trade will help in appreciating the role of veterinary preventive medicine and the imperative need for financial support for research in this field.

The prevention and elimination of diseases and parasites should be done by the techniques of preventive veterinary medicine. These problems of livestock with their bearing on leather industry cannot be simplified as mere biological problems.

There is no point in removing disease, or promoting health of livestock at enormous expense to the state, on mere humanitarian considerations, or if we plan to exploit our livestock for hides and skins only after their natural death, following starvation. If freedom from disease is established, a livestock production policy must be spelled out to harvest the products of livestock whereby it will be possible to remove any problems limiting better manufacture and improvement in quality of hides and skins.

Ticks Infesting Livestock in India, their Importance to Leather Industry and their Control

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About seventy different kinds (species, subspecies and varieties) of ticks are known to occur in India at present. Of these 5 species belong to the family Argasidae and the remaining belong to Ixodidae. Forty two kinds of these ticks are known to infest domestic stock. The names of these 42 kinds of ticks, the domestic animals from which these have been reported and their distribution statewise in the country are mentioned in this paper. The nature of the tick damage to hides and skins and the importance of ticks to leather industry in India are discussed. The results of a series of semifield trials conducted with some newer organophosphates (acaricides) against *Boophilus microplus* and *Hyalomma H.a. anatolicum* the two most important species of livestock ticks in India are given.

Carolus Linnaeus, the celebrated naturalist was the first to study ticks from India. He described two species of Indian ticks, one in 1758 named *Acarus elephantinus* and the other in 1767, *A. indus*. However, most of the ticks that we know to occur in India were described in the beginning of this century mainly by Prof. G.H.F. Nuttall and his associates. The first attempt to compile the records of ticks reported from the Indian Sub-continent together was made by Shariff[9] who published in 1928 " Revision of the Indian Ixodidae with special reference to the collection in the Indian Museum." He reported on 45 species, 4 subspecies and 6 varieties in 9 genera of Ixodid ticks from India ; but he did not include Argasid ticks in his study. Since then very little new information on Indian ticks came to light until very recently. Sen[8] has

published, "Check and host list of Ixodoidea occurring in India" and according to him 5 species of Argasidae and 39 species, subspecies and varieties of Ixodidae were known to occur in India . After the discovery of Kyasanur Forest disease in Mysore State in 1957 the workers at the Virus Research Centre, Poona, and their American associates, notably Dr. Harry Hoogstraal and Mr. G.M. Kohls, reported on a number of species of haemaphysalid ticks from India and described and about twelve new species of this genus. There is not the slightest doubt that the tick fauna of India is much richer than what it is known to be now and that much interesting information will come to light when a thorough study is made.

It is well known to many that ticks are harmful creatures, for they suck blood of animals and men as well and act as vectors and reservoirs of several important diseases. It is known only to a few that these parasites also cause great harm to one of the important commodities, a product of livestock and foremost foreign exchange earner for us, namely, hides and skins.

In recent years the study on Indian ticks has formed an important activity of the Division of Parasitology at the Indian Veterinary Research Institute, Izatnagar. Studies are being carried out on these ticks from the point of view of their taxonomy, life-history, habits, role in the transmission of livestock diseases and their control measures. In this article the different species of ticks infesting various kinds of livestock, their geographical distribution in India as we know to date and the result of experiments carried out for their control are described. The importance of these ticks to leather industry is also discussed.

Materials and Methods

For the last about twelve years ticks are being collected from the body of livestock and preserved in 70% alcohol. Though most of these collections are being made at Izatnagar ticks are also obtained from Veterinary field workers in different parts of the country for identification. These collections have been examined and their specific identification made in the light of the present day knowledge of the tick taxonomy. The data presented here have been based on the examination of the collections of the author and from other published works. Dr. Harry Hoogstrall and his associates have revised the names of a number of species of haemaphysalid ticks described by earlier workers such as Nuttall and Warburton[7], Shariff[9] and Sen[8]. The new nomenclature has been used in this article. In the description of the geographical distribution of the ticks the present boundary of the states in the country have been taken into account.

In the studies on the control measures against ticks reported in this article the new synthetic acaricides available were used. The chemicals in selected concentrations were sprayed on infested animals using hand compression sprayers at 50lb/sq. inch pressure. Comparative efficacy of different treatments were evaluated on the basis of the control of engorged adult ticks. Some of the results already published by the author have been included here with the idea that this will facilitate having all the information to gether in one publication.

Results and Discussions

Ticks Infestinng Livestock in India : So far a total of 70 different kinds (species, subspecies and varieties) of ticks are known to occur in India. Of these 5 species belong to the family Argasidae and the remaining to Ixodidae. Forty two kinds of these ticks are known to infest domestic stock. The names of these tick species, subspecies and varieties, the domestic animal hosts from which these have been reported and their distribution statewise in India are as follows : —

Name of ticks	Livestock from which reported	States from which reported
(1)	(2)	(3)
Family Ixodidae		
Genus *Hyalomma Koch*, 1844		
Hyalomma (*Hyalomma*) *anatolicum* Koch, 1844	Cattle, buffaloes, camels, mules, horses, pigs, sheep and goats.	Andhra Pradesh, Assam Delhi, Gujarat, Himachal Pradesh, Madhya Pradesh Madras, Punjab and Uttar Pradesh.
H. (*H*) *marginatum issaci* Sharif, 1928	Cattle, buffaloes, goats, horses, sheep, camels and dogs.	Andhra Pradesh, Bihar, West Bengal, Gujarat, Jammu & Kashmir Delhi, Madhya Pradesh, Mysore, Maharashtra, NEFA, Orissa and Uttar Pradesh.
H.(*H*) *dromedarii* Koch, 1844	Camels, Cattle, horses, goats and buffaloes	Andhra Pradesh, Delhi, Gujarat Maharashtra and Uttar Pradesh.
H. (*H.*) *detritum Schulze*, 1919	Horses, cattle, buffaloes, camels and sheep	Assam, Bihar, Delhi and Uttar Pradesh.

Name of ticks	Livestock from which reported	States from which reported
(1)	(2)	(3)
H (H) marginatum turenicum. Pomerantzev, 1946	Cattle and camels.	Gujarat.
H. (*Hyalommina*) *kumari* Sharif, 1928.	Goats, sheep, dogs, horses, buffaloes and cattle.	Assam, Bihar, Delhi, Madhya Pradesh, Maharashtra, Orissa, Punjab, Kerala and Uttar Pradesh.
H. (H) hussaini Sharif, 1928	Cattle, buffaloes, horses, goats, camels, sheep, and dogs.	Andhra Pradesh, Bihar, Delhi, Madhya Pradesh, Maharashtra, Mysore and Orissa.
H. (H). hussaini Sub sp. *brevipunctata* Sharif, 1928.	Cattle, buffaloes, goats, horses, camels and dogs.	Andhra Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Madras, Mysore, Kerala, Orissa and West Bengal.
Genus Boophilus Curtice 1891.		
Boophilus microplus (Canestrini, 1887)	Cattle, buffaloes, sheep, goats, horses and camels.	Assam, Andamans, Bihar, Himachal Pradesh, Gujarat, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Madras, Mysore, NEFA, Uttar Pradesh, West Bengal, Punjab and Orissa.
B. annulatus (Say, 1821)	Cattle and horses	Mysore.
Genus *Nosomma* Schulze, 1919		
Nosomma monstrosum (Nuttall and Warburton, 1908)	Buffaloes, cattle and dogs	Bihar, Goa, Orissa, Maharashtra and Uttar Pradesh.
Genus *Ixodes* Latreille 1795		
Ixodes ricinus Linn. 1758	Sheep and dogs	Himachal Pradesh and Jammu and Kashmir.
I. acutitarsus Karsch, 1880	Cattle	(NEFA) and West Bengal.

Name of ticks	Livestock from which reported	States from which reported
(1)	(2)	(3)
I. japonensis Neumann, 1904	Sheep and mules	NEFA and (Uttar Pradesh)
Genus *Amblyomma* Koch, 1844		
Amblyomma integrum Karsch, 1879	Buffaloes and cattle	Mysore and Orissa
A. testudinarium Koch, 1844	Cattle	Assam, Mysore, NEFA, and West Bengal.
Genus *Haemaphysalis* Koch 1844		
Haemaphysalis bispinosa Neumann, 1897	Cattle, buffaloes, goats, sheep horses, dogs, and cats.	Andhra Pradesh, Assam Andamans, Bihar, Gujarat Kerala, Maharashtra, Madhya Pradesh, Mysore, Madras, Nagaland, NEFA, Orissa, Punjab, Uttar Pradesh and West Bengal.
H. intermedia[12] Warburton and Nuttall, 1909	Sheep and goats	Maharashtra, Mysore and Orissa
H. paraturturis[6] Hoogstraal, Trapido and Rebello, 1963.	Cattle	Bihar
H. cornupunctata[4] Hoogstraal and Varma, 1962	Sheep, goats and yak.	Himachal Pradesh and Jammu and Kashmir.
H. kashmirensis[4] Hoogstraal and Varma, 1962	Goats	Jammu and Kashmir.
H. turturis Nuttall and Warburton, 1915	Cattle	Uttar Pradesh, (Kerala, Madras and Mysore)
H. sundari Sharif, 1928	Sheep	Uttar Pradesh
H. spinigera Neumann, 1910	Cattle and buffaloes	Madhya Pradesh, Mysore (Kerala and Orissa)
H. flava Neumann, 1897	Cattle and dogs	Madras and Uttar Pradesh

Name of ticks	Livestock from which reported	States from which reported
(1)	(2)	(3)
H. montgomeryi Nuttall, 1912	Cattle, sheep, goats and dogs	Himachal Pradesh Jammu and Kashmir and Uttar Pradesh.
H. leachi var *indica* Warburton, 1910	Cattle	Uttar Pradesh
H. cornigera Neumann, 1897	Cattle	NEFA
H. cornigera var *anomala* Warburton, 1913	Cattle and dogs.	Bihar
H. aponommoides Warburton, 1913	Cattle	West Bengal and (NEFA)
H. biramaniae Supino, 1897	Cattle	NEFA
H. neumanni Donitz, 1905	Cattle, goats, horse, sheep mithans and mules	NEFA
H. hystricis Supino, 1897	Cattle	NEFA
H. nepalensis[5] Hoogstraal, 1962	Sheep	NEFA[5]
Genus *Rhipicephalus*, Koch 1844		
Rhipicephalus haemaphyaloides, Supino, 1897	Buffaloes, Cattle, horses, goats, sheep, camels, mithans, and mules dogs	Andhra Pradesh, Assam, Bihar, Gujarat, Maharashtra, Madhya Pradesh, Madras, Mysore, Kerala, Punjab, NEFA, Orissa, West Bengal and Utter Pradesh
R. sanguineus sanguineus (Laterille, 1806)	Dogs, cattle, horses, donkeys and goats.	Throughout India.
Genus *Dermacentor* Koch, 1844		
Dermacentor auratus Supino, 1897	Horses	NEFA

Name of ticks	Livestock from which reported	States from which reported
(1)	(2)	(3)
Family Argasidae		
Genus *Argas* Latreille, 1796.		
Argas persicus Oken, 1818	Fowls	Throughout India
Genus *Ornithodoros* Koch, 1844		
Ornithodoros savignyi (Audouin, 1827)	Cattle	Gujarat, Madras, Maharashtra and Mysore
O. tholozani var *crossi* Desporates and Campana, 1946	Sheep and dogs	Jammu and Kashmir
O. lahorensis Neumann, 1908	Goats and sheep	Uttar Pradesh
Genus *Otobius* Cooley and Kohls, 1944		
Otobius megnini (Duges, 1884)	Horses	Madhya Pradesh.

* States where the tick has been recorded from animals other than livestock are given inside brackets.

However, the most important livestock ticks in India are *Hyalomma* (*Hyalomma*) *anatolicum, anatolicum H. H. marginatum isaaci, H. H. dromedarii, H. H. detritum, H.* (*Hyalommina*) *kumari, H.H. hussaini, Boophilus microplus, B. annulatus, Rhipicephalus haemaphysaloides haemaphysaloides, R. sanguineus sanguineus, Haemaphosalis bispinosa, H. montgomeryi, Ornithodoros savignyi* and *Aregas presicus.*

Importance of Ticks to Leather Industry in India : Ticks are next to only warble flies as the cause of arthropodal damage to hides and skins. This was the opinion of the hides Cess Enquiry Committee also, which stated in their report published in 1929-30 as follows. "A large body of opinion holds that next to the warble flies the parasite which cause most damage, both direct and indirect to hides and skins are ticks..... We cannot give separate figures in respect of tick damage, but we are convinced that the total loss must be enormous, and even the damage to hides and skins perhaps amounts to several lakhs of rupees per annum"[10]. Tick damage to hides and skins

is not peculiar to India alone. The effective use of acaricides against ticks have perhaps reduced their incidence in other countries, which faced similar problems earlier.

Ticks feed mainly on the blood of animals by piercing through the skin their mouth-parts, namely, the single hypostome and the paired chelicerae. The disintegration of the cells of the host's skin around the tick puncture under the influence of the saliva of hard ticks has been noted by various workers[11]. Some of the histopathological features of the skin around the inserted mouth-parts of feeding hard ticks include the so-called cement substance which encases the mouth-parts, the formation of haemorrhage and oedema of tissues. Thus, despite the host's normal wound healing process, after the tick withdraws its mouth-parts from the host's skin, a scar surrounded by a stain persists. When the leather produced from tick damaged hides or skins are finished, particularly if with grains intact, these scars and stains persist and thus the tanned leather is weakened and spotted. These scars and stains are very undesirable characters for otherwise a good leather and their presence depreciates its value to a great extent. When a large number of ticks feed on an animal the condition of the leather produced from such animal hide or skin can be well imagined. Thus considering the large number of tick population belonging to so many different species which infest livestock in this country it is not difficult to realise that the ticks are a very important factor contributing towards the enormous loss incurred by the leather industry in India.

Control of Ticks Infesting Livestock: In the last few years semifield trials with a number of newer synthetic acaricides against ticks infesting our livestock have been carried out. The tick species against which these tests have been conducted are B. *microplus* and *H.* (*H*) *a. anatolicum.* the two most common species found to be infesting cattle in this country.

In one of these tests diazinon at 0.5%, and lindane at 0.25% were used. A group of six hill bulls infested with the tick *B. microplus* were treated with each treatment ; another group of six such bulls was treated with plain water as controls. All the cattle treated with these insecticides excepting those treated with toxaphene were found to be completely free from infestation with adult female ticks when examined two days after treatment. The action of malathion and DDT mixed with lindane was quicker than that of diazinon. Malathion and diazinon treatments kept the cattle free from attack of adult female ticks for not less than eight days and not more than 14 days but the treatment with DDT mixed with lindane protected the cattle from adult female ticks upto a minimum of 29 days. This showed that the former two insecticides did not make the treated animals completely free from larval and

nymphal stages of the tick, perhaps those in the process of moulting survived, whereas the latter treatment did so. Treatment with toxaphene did not give satisfactory result under the condition of this test.

In another test coumaphos and trichlorphon at three concentrations *viz*. 0.125%, 0.5%, and 0.75%, fenchlorphos at 0.5% and 0.75% and DDT at 0.25% and 0.5% were used against the same species of ticks, (B. *microplus*). A group of three Fresian cross-bred dry cows heavily infested with this tick were treated with each drug and a group of three cows was treated with plain water served as controls. Four days after treatments animals treated with all the different concentrations of the insecticides used except for those treated with 0.25% DDT were practically free from the attack of the adult female ticks. The activity of all the three different concentrations of trichlorphon and of 0.75% coumaphos were quicker than that of others. Coumaphos and trichlorphon at 0.5% and 0.75% and DDT at 0.5% removed the larval anp nymphal stages of the ticks as well from the body of the treated cattle. The effect of the treatments other than those with 0.75% coumaphos, all the three concentrations of trichlorphon and 0.5% DDT against the seed ticks did not last longer than 10 days but in the case of the above treatments it lasted at least for a fortnight. None of the engorged female ticks dropped off the cattle treated with 0.5% and 0.75% coumaphos and 0.5% DDT and very few of those from cattle treated with 0.125% coumaphos and all the three concentrations of trichlorphon laid eggs. For detailed informations on these tests reference may be made to the published paper[1].

In one of these tests coumaphos was tested against the tick *H.* (*H*) *a. anatolicum* infesting buffaloes. A herd of buffalo calves, about six months old, were found to be very heavily infested with thousands of this tick species. The tick population consisted of all the different parasitic phases of its life cycle and the females were not fully engorged. The tick infestation was so heavy that the animals appeared sick and some of them had already succumbed to it. Ten of these animals were treated with 0.15 % and another 10 with 0.25% coumaphos. Twenty four hours later the tick population on all the treated animals was found to have gone down by about 60%. Most of the ticks were found to have dropped off the hosts but a few were still found attached either dead or moribund, on the hosts. Samples of live ticks were collected from the ground around the treated animals and kept in the laboratory for observation. During the next 24 hours many of these ticks died and the rest except a few in each group were moribund. Even the engorged female ticks were also found to have been killed by both the treatments and no appreciable difference was noticed between the effect of 0.15% and 0.25% coumaphos treatments against this species of ticks.

Five days after the first treatment the two groups of buffalo calves were retreated with the respective drugs. Twenty four hours after this second treatment it was observed that the number of ticks on the treated calves was reduced to 5-7%[2].

In another test, the efficacy of two newer organophosphorus compounds of low mammalian toxicity were used against the tick B. *microplus*. The acaricides used were (1) "Nankor," a product of Dow Chemical International Ltd., U.S.A., and (2) "Bercotox" a formulation containing 30% delnav and a product of William Cooper and Nephews Inc. Chicago, U.S.A. The first acaricide was used at 0.5% and 0.75% concentration and the second at 0.075% and 0.15%. Coumaphos at 0.5% concentration was used as control. Five Fresian Cross-bred cows infested heavily with *B. microplus* ticks were treated with each of the concentrations of the acaricides and five cows were treated with plain water as controls. The results are summarized in Table 1.

TABLE 1

The nature of control of engorged female tick *Boophilus microplus* on cattle treated with Bercotox, Nankor and Coumaphos.

Insecticides and % conentration.	Average number of engorged female ticks per animal before treatment.	% Control of engorged female ticks per animal on different days after treatment.			
		2	7	21	35
Bercotox 0.075	68.2	92.8	98.28	92.9	4.17
Bercotox 0.15	103.6	91.4	100.00	100.0	47.33
Nankor 0.5	76.4	79.4	89.42	61.21	37.78
Nankor 0.75	89.0	92.6	99.36	98.91	26.38
Coumaphos 0.5	68.6	91.0	100.0	100.0	30.56
Control	98.4	—	—	—	—

Two days after the treatments were applied both the concentrations of "Bercotox" 0.75% 'Nankor' and 0.5% coumaphos were found to be more or less of equal effect against the tick each giving about 91–92% control of adult female population. Nankor at 0.5% was inferior to these and gave only 79.4% control of adult female ticks. Later when the experimental animals were examined one and three weeks after treatment practically no adult

tick was found on any of the animals treated with acaricides except those treated with 0.5% Nankor whereas on an average 92.6 and 36.6 female ticks per animal were found on these occasions respectively on the controls. Not much difference was noticed in the tick population on different groups of animals whether treated with acaricides or plain water, when examined five weeks after the treatments.

Results of these tests indicate that there are a number of acaricides, some of which are available in the market in this country, by the proper use of which the tick infestation on livestock can be controlled and thereby, besides protecting the animals from the other kinds of harm caused by ticks hides and skins of these animals can be protected from tick damage.

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A Study of the Ticks of the Madras State with Special Reference to their Importance in Leather Industry

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The contributions of Patton and Cragg (1913), Sharif (1928) Sen (1938), Nagar (1962.), Hoogstraal (1956), and Kaiser and Hoogstraal (1964), and others are reviewed. Species of *Argas, Ornithodorus, Hyalomma, Amblyomma, Haemaphysalis,* and *Aponomma* have been studied with regard to their identity, in the light of recent taxonomic revisions, and their occurrence in the Madras region.

Argasid and Ixodid ticks cause direct injury and trauma to the skin followed by exsanguination, and impair the health of livestock by anaemia, tick worry and toxicosis and by transmission of microbial pathogens. The process of feeding is related to the functions of the chelicerae, hypostome and other oral armature, involving considerable liquefaction of tissue and absorption of blood. Skin damage would depend on the pattern of mouth parts, feeding habits, and zonal distribution of the different species of ticks.

Though the role of ticks as agents of disease in man and animals has long been recognized in India, the tick fauna in the country as a whole and in the Madras State in particular have not received the attention they deserved. Sharif[40] considered that Indian species were "poorly described and insufficiently illustrated," and this comment is fully tenable even at the present time, not withstanding the great advances made elsewhere in the world in regard to their taxonomy, morphology, life-cycles, and transmission of microbial diseases.

The present account aims to review the knowledge about the ticks in Madras State, with a view to highlighting the status of existing information, and the scope for and the trend of future researches towards improving animal health, and leather industry in particular. The economic importance of the ticks lies in the fact that they can inflict harm on the animals directly and indirectly. Direct injury will result from their "bites," leading to destruction of skin tissue, exsanguination and anaemia. The indirect consequences of such parasitism would include tick worry and toxicosis, on the one hand, and transmission of protozoal, bacterial, viral, and rickettsial pathogens, on the other.

Review of Literature

The earliest collections and studies of ticks in Madras were those of Neumann[21-25], Christophers[9,10], Warburton[46], and Patton and Cragg[31]. Nuttall and Warburton[28,29,30], Robinson[36], and Arthur[4] contributed monographs on the Ixodoidea in which the various genera and species occurring in India were reviewed. Sharif[40] revised the Indian Ixoididae and furnished keys to the different species of Indian ticks. The following species were reported by him as occurring in Madras :—

Haemaphysalis flava, H. turturis, H. bispinosa, H. b. var. *intermedia* (= *H. intermedia*), and *H. spinigera, H. leachi* var. *indica, Rhiphicephalus sanguineus, R. haemaphysaloides, Boophilus australis*) (= *B. microplus*), *Hyalomma aegyptium* forma typica (= *H. anatolicum anatolicum*), *H. aegyptium* s.sp. *isaaci* (= *H. marginatum isaaci*) *H. hussaini, Amblyomma integrum, Aponomma gervaisi,* and *A. laeve.*

The two species of Argasid ticks, *Argas vespertilionis and Ornithodorus savignyi,* mentioned by Patton and Cragg[31] had not, however, been incorporated in the revision undertaken by Sharif[40]. *O. savignyi* was collected and utilized in experiments with *Trypanosoma evansi* by Rao and Ayyar[32]. Sen[37] undertook the preparation of a checklist of the Ixodoidea in India and appended a host list. According to him *O. savignyi,* recorded by Christophers[9] was the only species of the Argasidae in South India. The members of the Ixodidae reported from South India were :—

Haemaphysalis flava, H. turturis, H. bispinosa, b. var. *intermedia, H. spinigera, H. leachi* var. *indica, Hyalomma aegyptium, H.* (*Hyalommina*) *hussaini, Amblyomma integrum, Aponomma gervaisi* and *A. laeve.*

Sharif[41] appended a list of Indian ticks on domestic animals to his account of tick-borne dieseases.

Alwar[3] studied the incidence of 14 species of the subfamily Amblyomminae, in various districts of Madras, and from several hosts, inclusive of a new species *Amblyomma mudaliari* reported by Rao, Hiregaudar and Alwar[33] from cattle and buffaloes, and subsequently[34] described by them. *Dermacentor auratus* from a cow, *Amblyomma integrum* from cattle and buffaloes, *A. testudinarium* from hyaena, nymphs of *Amblyomma* sp. from elephants, *A. gervaisi* from *Varanus*, *A. pattoni* from cobra and cattle, *H. leachi* var. *indica* from a cow, panther and a mongoose, *H. parva* from sheep, cat and hare, and *H. turturis* from a barking deer, were additions to the list of known species.

Nagar[26], and Kaiser and Hoogstraal[18] revised the genus *Hyalomma* and commented on certain characters of specific value. The latter authors considered that "taxonomically, the endemic species have been inadequately described and delineated," and discussed the specific status of some of the South Indian species.

In their text book on Veterinary Entomology and Acarology for India, Sen and Fletcher[39] provided a taxonomic account of the Indian species with notes on their biology, summarizing the various publications on the subject.

At the suggestion of the author, investigations were initiated in this department with a study by Kamath (unpublished dissertation, 1967) on Ixodid ticks of cattle, sheep and dogs in Madras City. He found *Boophilus microplus* as the commonest tick on cows in a Dairy Farm and *Rhipicephalus haemaphysaloides*, *Hyalomma marginatum isaaci* and *Haemaphysalis bispinosa* less commonly. Variations and malformations in the adanal and accessory plates in the males of *B. microplus* were studied in detail. From sheep in the slaughter house, *Haemaphysalis intermedia*, *H. bispinosa*, *Hyalomma marginatum isaaci*, *Rhipicephalus haemaphysaloides*, and *Hyalomma* (*Hyalommina* sp.) were encountered. All stages of *H. intermedia* were found on the same animal, and nymphs of *H. m. issaci* were reported as of a rare occurrence. *R. sanguineus* and *H. intermedia* had been found to infest dogs.

Remarks on Genera and Species

1. *ARGASIDAE*

1. *Argas* : *A. vespertilionis* — on yellow Bat (*Scotgphilus kuhli*) — S. India, Patton and Cragg[31] ; Arthur [7] refers to it as *A.* (*Carios*) *vespertilionis* though it is not found in the lists of Sen[37], Sen and Fletcher[39].

A. persicus — North and Central India only, Patton and Cragg[31] ; " only species in India " Sen and Fletcher[39], life history studied by Cross and Patel[11] in Punjab. This species has not been encountered in Madras so far, but an intensive search may reveal its presence in restricted areas.

2. *Ornithodorus* : *O. savignyi* — Neumann[29b]. Christophers[9] ; Patton and Cragg[31] ; Rao and Ayyar[32] ; Sen and Fletcher[39].

Its presence in South India was recorded by these authors and reported as common in Madras Presidency, in railway waiting sheds and under large trees, and its life history studied[31]. The present author has obtained large numbers of these from the goshala in Tiruchendur, Ramnad District, on several occasions since 1962, and maintained them in the laboratory by experimental feeding on dogs. Feeding lasted for about thirty minutes and was accompanied by a sanguineous exudate and inflammation of the area.

3. *Otobius : O. megnini* - was first recorded in India by Kingston[19] from horses in Sagar, Madhya Predesh, and the life history studied by S.K. Sen[38]; but its occurrence in Madras is not reliably known.

2. *IXODIDAE*

4. *Ixodes :* Though four species have been reported from India, none of them was found in South India or Madras.

5. *Haemaphysalis : H. flava. H. bispinosa, H. b.* var. *intermedia, H. spinigera, H. parva and H. leachi* var. *inaica* in Madras[3,30,39,40]. *H. turturis* was first recorded in Parambikulam in Cochin, and later in Topslip, Madras[3]. Patton and Cragg[3], mentioned two unidentified species, one on the South Indian hare *L. nigricollis* and the other on the Gerbil, *Gerbillus ind'cus* as occurring in Madras. A useful guide for the identification of all stages of *Haemaphysalis* in South India is now available[44] as well as a description of all stages of *H. turturis* by Hoogstraal and Varma[17].

6. *Rhipicephalus* : The two species, *R. sanguineus* — the commonest Dog tick (Guindy, Madras; Warburton[46]), and *R. haemaphysaloides* occur in the region[31] ; the latter species was collected from Cuddalore, South Arcot District and Srivilliputtur, Ramnad district[40] ; from cattle and sheep in Madras City and elsewhere (present author.) Hoogstraal (personal communication, 1968) suggests that as *R. turanicus* replaces R. *sanguineus* in many parts of India, *R. sanguinens* from Madras may be checked against the descriptions of the other species.

7. *Boophilus* : Patton and Cragg[31] reported *Margaropus annulatus* var. *australis* from Madras, which was synonymized with *B. australis* (Sharif[40]) and considered to be very common, along with *Hyclomma aegyptium*,on cattle in Madras (Sen and Fletcher[39]). *B. microplus* ($=B.$ *australis*) occurs on cattle in Madras City (Kamath, loc. cit.) and outside as well (present author).

8. *Dermacentor* : The only Indian species *D. auratus*, known from wild animals in Assam, Bengal, Orissa, Uttar Pradesh and Bombay ;—first record in Madras on a cow in Coimbatore[3].

9. *Amblyomma* : Patton and Cragg[31] listed three species in South India: *A. crenatum, A. prolongatum, A. testudinarium. A. integrum* from Guindy and Saidapet[36,37,40], in Sivagiri, Madras[3]. Further records include *A. mudali'ari* (cattle and buffaloes), *A. testudinarium*, (Hyaena — Coimbatore), and *A.* sp., (elephant) by Alwar[3].

10. *Hyalomma* : *H. aegyptium* was the only species mentioned by Patton and Cragg[31], as being widely distributed on cattle, buffaloes, horses, dogs and the South Indian hare *Lepus nigricollis* ; larvae on the Palm squirrel and the hare. *H. aegyptium* occurred on horses in Madras City[45]; Sharif[40] regarded Patton and Cragg's material as consisting of *H. aegyptium forma typica* and *H. ae. dromedarii* in parts. A new subspecies, *H. ae. isaaci. H. hussaini* n.sp. and *H. kumari n.sp*[40,41]. were from Chittoor District, Krishna District, and Cochin in South India respectively, but beyond the limits of the present Madras State.

Feldman - Muhsam[13,14,15], Hoogstraal[16], Nagar[26,27] and Kaiser and Hoogstraal[18], revised the taxonomy of this genus and the Indian species, according to which *H. marginatum issaci* is the only species from Madras. Ticks from cattle in the Madras City and elsewhere in the State have been identified as *H.m. issaaci* by Kamath (loc. cit.) and the author.

11. *Aponomma* : Two species, *A. gervaisi* (from *Zamenis mucocus* and *Varanus bengalensis*) and *A. pattoni* (from *Z. mucocus*) reported by Patton and Cragg[31]. The occurrence of *A. pattoni* in Saidapet, Vellore District[24], and *A. laeve* in Coimbatore, Madras, were mentioned by Sharif[40]. Sen[37] listed A. *gervaisi* (in the whole of India) in addition to *A. laeve*. Reference to all the three species was made by Alwar[3].

In a recent survey of ticks from birds and small mammals in North Arcot and Chittoor Districts, South India, Rebello and Reuben[35] reported the following Ixodid ticks :

a) *Haemaphysalis intermedia, H. bispinosa* and *H. marginatum isaaci* from 136 birds belonging to 16 species, all in North Arcot, Madras ;

b) *Hyalomma brevipunctata, H. sp* (?), *Haemaphysalis leachi* group, *R. haemaphysaloides* and *R.sp*? (3) from 121 rodents and insectivores, in both Andhra and Madras area ;

c) *H. brevipunctata, H.a. anatolicum, H. intermedia, H. leachi* group and *R. haemaphysaloides* from dog, mongoose, hare, pig, man and on soil, all in North Arcot, Madras ; and

(d) larvae of *Ixodes ceylonensis* from *Rattus r. wroughtoni* and *Suncus murinus* in Ootacamund, Madras.

In addition to the species in Madras City reported from this Department (Kamath, loc. cit.), the following have been collected from certain localities in the State, during the last four years, and identified as :- *O. savignyi* (Tiruchendur), *R. sanguineus* (dog, cow— Madras city and Alamadhi farm), *R. heamaphysaloides* and *R. sp.* (sheep), *H. bispinosa* (heifer, sheep — Madras City and Coimbatore respectively), *H.m. isaaci* (= *H. aegyptium*) (Sheep and goats — Kattupakkam; Ramnad), *Hyalomma* sp. (ewe — Chinnadarapuram) and *B. microplus* (cow, bullock — Melur and Chinnamannur). Males and females of *R. haemaphysaloides R. sanguineus*, and *H. bispinosa* from the hare *Lepus nigricollis*, and *R. haemaphysaloides* from the jackal have been encountered at the Alamadhi cattle farm near Madras.

Among all the above genera, *Boophilus* and *Hyalomma* are of much economic importance infesting, as they do, cattle, sheep and goats. Their identity and taxonomy have been re-investigated in detail by recent workers ; Arthur[4] has revised the genus *Boophilus* and maintained *B. microplus* as a common tick on cattle and to include *B. australis* and *Margaropus annulatus* of earlier authors.

In regard to *Hyalomma*, Adler and Feldmann-Muhsam[2] appear to have revived *H. savignyi* Gervais (1844) and synonymized *H. aeygptium* of Patton and Cragg[31] and subsequent authors, with which Sen and Fletcher[39] agreed. Feldman-Muhsam[13] held that " upto 1930 most authors referred to any *Hyalomma* as *H. aegyptium*" and that *H. aegyptium* did not attack domestic stock. She agreed with Hoogstraal[16] that Sharif's[40] *H. aegyptium f. typica* was *H. excavatum*, and considered *H. hussaini* and *H. kumari* of India

as valid[14] and *H. anatolicum* as *species inquirenda* with *H. a. anatolicum* and *H.a. excavatum* as one polymorphic species.

In his examination of ticks from Delhi State, Nagar[26] confirmed the validity of *H. hussaini* and *H. kumari* and supported Feldman - Muhsam's[13] view that *H. aegyptium* of Sharif[40] was a different species.

The *Hyalomma* of Pakistan, India and Ceylon was reviewed by Kaiser and Hoogstraal[18] maintaining three subgenera, *Hyalommasta*, *Hyalomma* and *Hyalommina*. According to them, *H. aegyptium* is the Tortoise species from Pakistan, and all other records of it refer to *H.a. anatolicum*, *H. dromedarii* and *H. marginatum s. sp.* There other species *H. kumari*, *H. hussaini* and *H. brevipunctata*, from Parambikulam, Chitoor and Anantapur repectively, cannot now be assigned to Madras State, which leaves *H. marginatum issaci* as the only species native to the State. It should be agreed with them that "the biology and ecology, as well as the Medical and Veterinary importance of all *Hyalomma* ticks, in the Indian subregion, is insufficiently known."

Biological and pathological aspects of tick infestations

The relationships of tick infestation to the causation and transmission of diseases in livestock have been brought into focus by Sharif[41], Abdussalam[1] and Arthur[5,6] but scarcely any evidence obtained regarding the loss of skin quality in the affected animals.

The direct injury to the skin is however dependent on the feeding processes and feeding patterns and ecology of the Argasid and the Ixodid ticks. All the stages of the Argasid ticks and the free-living stages of the *Ixodid* ticks are under the influence of such environmental factors as temperature, rainfall and humidity, vegetation, and texture of soil. The microclimate around these stages requires to be investigated critically. The feeding pattern varies greatly from the one-host to the three-host association, species of *Boophilus* group adopting the simplest behaviour of being restricted to one host, the genera *Amblyomma*, *Haemaphysalis and Ixodes*, choosing three successive hosts for the immature and adult stages, and species of *Rhipicephalus* showing the greatest variation in feeding behaviour with two or three hosts. Such host preferences and ecogeographical aspects of tick distribution in Africa have been studied by Theiler[43].

The mechanism of feeding in various ticks studied in recent years has been reviewed by Arthur[8]. While Argasid ticks generally feed for short

periods ranging from a few minutes to hours, but several times (4-7 in *O. savignyi*), Ixodid ticks persist in a feeding state for several days. The soft tick also penetrates the skin rapidly and actively whereas the hard tick resorts to a more deliberate lacerating action followed by attachment for a longer duration. This would be reflected in the skin receiving several minor punctures, in one case, and in a more serious trauma, though in restricted areas, in the other. The haemorrhage produced by the soft ticks is diffuse and superficial and infiltrates into the dermal layer. The rapid induction of the haemorrhage suggests quick lytic properties of the salivary secretions.

The Ixodid tick presents a different process attended with trauma of a more lasting nature. The initial laceration is achieved by the cheliceral claws, leading to rupture and displacement of epithelial cells. The hypostome penetrates the wound as the chelicerae continue to work in, and the depth to which it can penetrate is related to the length and protraction of the chelicerae: larval forms and adults of *Haemaphysalis* and *Boophilus* (brevirostrate) reach only superficial layers, while in Ixodes (longirostrate type) the hypostome is buried deep in the dermis. The salivery secretions cause cytolysis in the deeper layers of the skin, followed by the suction of such liquefied tissue. The impression that ticks are exclusive blood feeders is no longer tenable, as significant amounts of tissue constitute their feed, through a tick may suck 2 to 6 times the quantity of blood it retains in its body. Evidence from the extensive breakdown of Mast cell granules accompanying the invariable accumulation of tissue fluid in the microtrauma points to the fact that considerable quantities of lysed tissue could be ingested during an extended period of slow feeding resulting in excavation inside the skin matrix.

Probing into the feeding processes of *Boophilus microplus*, Moorhouse and Tatchell[20a] made some interesting observations. Penetration of the mouth parts never reached below the base of the Malpighian layer even after 2 days, the hypostome being inserted only within the epidermis (Malpighian layer and stratum corneum) 50 μm thick, and most of the armature being imbedded in the "cement." They found considerable variation in the depth of penetration of the mouthparts of the different ticks : while those of adult *Dermacentor sinicus*, *Ixodes ricinus* and *Aponomma undatum* were fully inserted in the skin, in *B. microplus* they were shorter and yet superficially inserted, *i.e.* not below the epidermis. Nortwithstanding differences in size, the mouthparts of the larva, nymph, and adult *B. microplus* penetrated the skin to the *same* depth contrary to an earlier view[8] that the depth increases with the successive developmental stages. Heavier loads and a higher proportion of maturing ticks were found on the European breeds of cattle than on the Zebu.

The depth of penetration or the rupture of a blood capillary may not bear an absolute relation to the brevirostrate or longirostrate nature of the mouth parts, but will depend on the varying angles at which they enter the skin. What physical factors, if any, determine the angle could only be speculative : hairiness or otherwise, texture, temperature, etc., of the skin.

A zonal distribution of various species of ticks on the body of a host (cattle, sheep or goat), or of the developmental stages of any one species of tick may also be conditioned by similar factors (besides odour, etc.) For instance, O. *erraticus* chooses its feeding sites among hairless or sparsely hairy regions of the body[12], and on sheep *I. ricinus* occurs in largest numbers on the head, and in lesser proportions in the axilla and groin, with about 16% of the population under the long wool in the neck, chest and shoulders[20]. Larvae and nymphs of *I. ricinus* are distributed on the tips of ears, around the nostrils, chin, fetlock and axilla of sheep. On cattle, however, the same species is heaviest on the forelegs and in the axilla, and in smaller numbers in the groin, on the udder, flank and dewlap. The importance of mapping the predilection sites for each species of tick and host in relation to skin quality cannot be over-emphasized.

It is perhaps not fully realized that besides cattle, sheep and goats, the skin of rabbits, hares and reptiles are utilized in leather-craft. The tick fauna of hares, and reptiles (Python, Cobra, Russell's viper, Rat-snake, *Varanus*, etc.) in Madras need to be surveyed and their biology studied.

Kaiser and Hoogstraal[18] remarked that "the relationships of the Indian *Hyalomma* fauna to dissemination of diseases are, however, almost entirely unstudied. Biological and ecological data concerning these ticks are conspicuous by their extreme dearth." This is true not only of *Hyalomma* but of most other genera in India, except perhaps *Haemaphysalis*, which has been intensively studied at the Virus Research Centre, Poona, in the wake of the outbreaks of Kyasanur Forest Disease in Western India. Knowledge about the Ixodoids of tropical Africa has progressed in immense proportions, in contrast, from the studies of Yeomen[47] in East Africa, Hoogstraal[16] in the Sudan, Theiler[42] in the Ethiopian Region (South of the Sahara), and Yeomen and Walker[48] in Tanzania, models of scientific endeavour to be emulated in India.

This paper would have fulfilled its purpose if it stimulated workers to initiate tick surveys in localities of Madras State as starting points for future extensive studies. Hoogstraal (1967, personal communication) expects that " the Madras area should be rich in new information on ticks and tickborne diseases."

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The Role of Longirostrate Ticks in the Causation of White Spots in Sheep and Goat Skins

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The present work brings to light that longirostrate ticks are responsible for white spot defects produced on the flesh side of sheep and goat skins. The depth of penetration and associated degenerative changes caused in the tissue contribute to the causation of this defect, since these changes were restricted to infestation with longirostrate ticks (*Hyalomma* sp.) ; they were not evident in the deeper layers of the skin infestation in the case of brevirostrate ticks (*Haemophysalis* and *Boophilus* sp.)

The role of ticks in human economy merits special consideration not only because they are annoying pests in temperate and tropical countries but surpass all other arthropods in number and variety of diseases they transmit to man and domestic stock[1]. The importance of ticks as carriers and intermediate hosts is well known. With the advancement of the leather industry, the need for flawless raw materials has been felt more keenly now, and ticks constitute a major contributory factor in deteriorating leather quality. Among the defects of biological origin, "white spots" seen on the flesh side of tanned leather are particularly detrimental to suedes, which suffer considerably in their export potentiality. The causation of white spots in particualr reference to ticks is discussed.

The harmful effects caused by ticks have been referred to as early as 200 B.C. by M. Porcius Cato[1]. The economic importance and control of ticks have been extensively reviewed [2, 3, 4, 5, 10, 11, 18, 19, 24, 25, 28]. Work on ecographical and microclimatic conditions on animals have been alluded to by Theiler[20] and Arthur[3]. Studies on the anatomy of the mouth parts and biting mechanisms have been made extensively [1, 2, 3, 6, 12, 15, 18, 21, 25, 26]. The cement substance as contributed by the host tissue and the tick as well as the reactions caused by the saliva of the ticks, are subjects of great controversy and interest [1, 8, 9, 13, 20, 23, 27]. The histology and histopathological changes produced by tick bites have been widely reported. The works of Yutaka and others,[31] Arthur,[3] and Riek[23] give a detailed review of the knowledge in this field. Kusel 'tan[17] has described the skin reaction in lambs produced by *Hyalomma asiaticum* in connection with infection with *Nocardia* and causation of polyarthritis in lambs.

It is surprising that the association of such changes in direct relation to the damage and type of defect caused on the skin is vague except in a few instance where tick bites have been directly associated with damage to the quality of skins [4, 5, 10, 11, 8 14, 22, 24, 26, 28, 29].

Particular work with reference to the depth of penetration of the skin in relation to the damage produced in the leather, and the group of ticks involved does not appear to have been undertaken.

A study of the leather defect in relation to the depth of penetration by longirostrate and brevirostrate ticks has therefore been made.

Materials and Methods

Sheep and goat skins were obtained from known animals, after antemortem examination in slaughter houses and farms. Skins with ticks *in situ* were obtained and appropriately marked. Depending upon the nature of investigation made, studies were directed on the defects seen on the flesh side of raw skin, tanned leather, as well as in materials fixed in formal-saline to determine the tissue changes. In the tanned skins defects produced in the leather were noted down after processing. The ticks present were collected and identified with reference to their brevirostrate (*Haemaphysalis* and *Boophylus* sp.) or longirostrate (*Hyalomma* sp.) armature. The observations made are presented here.

Results and Discussion

Brevirostrate ticks : Seventy sheep skins and fifteen goat skins were obtained from the slaughter houses and farms with the brevirostrate ticks *in situ*. No lesions or white spots as such were observable on the flesh side. These skins did not on tanning, reveal any white spots on the flesh side in areas where the brevirostrate ticks were found to be attached in the raw skin.

Sections of brevirostrate ticks *in situ* in raw skin reveal that the mouth parts are embedded superficially penetrating the epidermis and the grain layer to some extent.

It would be seen in Fig. 1 that there is a layer of homogeneous eosinophilic cement substance on the epidermis on either side of the chelicerae, and the inflammatory reaction is oberservable. There is infiltration of eosinophils and neutrophils and occasional giant cells. Necrotic and degenerative changes are not marked, and extravasation of erythrocytes is also minimal.

Fig 1. Brevirostrate tick *in situ*. (Sheep skin)

Longirostrate ticks: Twenty sheep and six goat skins with longirostrate ticks *in situ* were obtained. The raw skin revealed a pale red spot on the flesh side corresponding to the point of attachment of the tick on the grain side. A congested area in the form of a circular lesion could be observed in some cases on the flesh of the skinned carcass corresponding to the site of the lesion.

These skins were found on tanning to show a white spot in these areas revealed by a paler shade. These areas were also found to have a poorer affinity for acid and basic dyes and stood out as lighter spots in dyed leather as well.

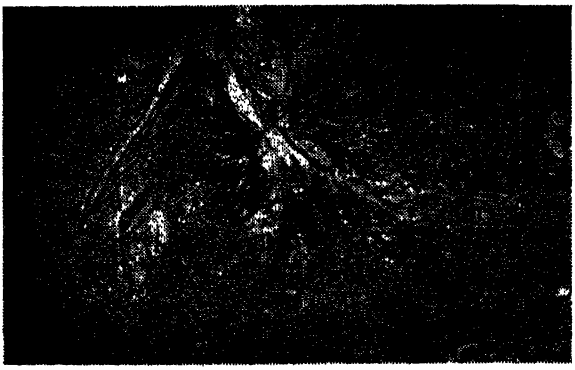

Fig 2. Longirostrate tick *in situ*. (Sheep skin)

It would be seen from the section of the longirostrate tick *in situ* in sheep skin (Fig. 2) that the mouth parts have penetrated almost the entire thickness of the skin. The cement substance is present, as in the brevirostrate tick lesion, but the eosinophilic tint of the cement substance seems to have pervaded deeper into the tissues. The inflammatory reaction around the trauma was characterised by inter and intracellular oedema, and leucocytic infiltration consisting of lymphocytes, eosinophils and polymorphs. The presence of the former cells suggest the presence of chronic inflammation of a parasitic nature. The lesion involved a conical area of tissue, with the base located at the flesh side of the skin and the apex located at the point of penetration of the grain layer of the skin. The mouth parts of the tick were also surrounded by a layer of eosinophillic structurless mass. Capillary rupture and extravasation are copious and free erythrocytes are present in the deeper layers of the dermis.

In the live animal inflammatory oedema of the skin is clearly visible beneath the hair. Such changes are faint in the case of brevirostrate ticks and in the larval and nymphal forms of both groups of ticks.

The comparative differences in the lesions produced by brevirostrate and longirostrate ticks in skin tissue is summarised in Table 1.

TABLE 1

Brevirostrate and longirostrate lesions in skin tissue

Description	Brevirostrate	Longirostrate
Depth	Superficial	Deep
Structures involved	Epidermis and superficial layers of dermis only	Epidermis, dermis, extending up to the subcutis
Inflammatory reaction	Slight, deeper, parts of dermis not significantly affected	Trauma with severe necrotic and degenerative changes. Involves deeper layers and extends to the subcutis
Cellular change	Slight	Marked. Derangement of tissue architecture and leucocytic infiltration

The causation of skin defects due to tick bite is well-known and has been widely reported [4, 5, 10, 29]. Lavoipierre and Rick[20] classified the argasid ticks according to the microscopic and macroscopic changes they cause on the host skin of experimental animals. Kuesl' tan[17] has discussed the influence of the longirostrate tick *Hyalomma asiaticum* on lambs, with particular reference to the production of polyarthritis. The present report classifies the defect caused on the animal skin with references to the length of mouth parts and their depth of penetration into the skin. It is obvious that the scope for total penetration is greater in the thinner areas of skin such as the shank and belly and may account for greater frequency of occurrence of white spot defects in those areas. The fact that the grain damage is far less obvious than the flesh damage indicates that the secretions and excretions of the tick and the tissue reactions of the host play a vital role in the causation of this condition. It must be mentioned that white spots in goats are also caused by Demodicosis but the aetiological factors and tissue changes differ significantly[7].

In a recent study of Ixodid ticks[16] it is reported that the longirostrate ticks of the species *Hya'onma marginatum isaaci* are commonly found in Madras. The prevalence of white spots in sheep and goat skins appears to confirm this. Longirostrate ticks are further found to be distributed in the shanks and belly and white spots in these regions are more common than in other parts of the body of sheep.

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Attempts in the Control of Bovine Hypodermosis with Several Non-organophosphorous Compounds Used in Veterinary Practice

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During the last three years, several non-organophosphorous compounds, which are classically used in veterinary practice against intestinal parasites, have been tested against the first and second stage larvae of *Hypoderma bovis* or *H. lineatum* during their migrations.

Phenothiazine, Methyridine, Carbamazine and Tetramizole in injections, Thiabenzadole and Nivaquine per os at the usual dose were ineffective.

Using several organophosphorus compounds, mostly administered by percutaneous route and pour-on method, it is now possible to destroy first and second stage *Hypoderma bovis* and *H. lineatum* larvae during the period of migrations inside the body of the host.

Among them, Ruelene (trade mark for 0-4 terbutyl 1-2 chlorophenyl 0-methyl methylphosphoramidate), Trichlorfon (common name for 0-0-dimethyl 2-2-2-trichloro-1-hydroxyethylphosphonate), Fenthion (common name for 0-dimethyl 0 (4 (methylthio) m-tolyl) phosphorothioate and Sisvar (trade mark for N-methyl 5-0-0 dimethyldithio phosphory l -3-thia-2-2-dimethyl valeramide) are frequetly used in France and Western countries.

In spite of the easiness of the pour-on method, based on the high skin penetrating power of a large number of organophosphorous coumpounds, the eradication of warble fly remains difficult because farmers do not correctly estimate the real damage caused to agriculture by this parasitism and are not inclined to make the effort and spend the necessary time and money on the treatment.

For this reason, it may be logically derived that a drug which would be effective not only against hypoderma larvae but also against internal, and specially intestinal parasites would be of course of great importance because farmers generally pay more attention to such infestations.

The purpose of the present report is to relate the trials conducted in our experimental centre, to verify the eventual effectiveness against hypodermosis of classical drugs used in veterinary practice, especially on cattle. All these efforts did not prove successful but it would be interesting to know with which drugs these experiments were conducted.

Phenothiazine orally administered for decades against intestinal worms has been proved ineffective for warble fly control, after several contradictory results and discussions[1]. Moreover we found that it was ineffective even as injections at the single dosage of 20 mg/Kg [2].

The other medicaments that we experimented unsucessfully [2,3] for warble fly control, were

Methyridine (common name for 2-(β.-methoxyethyl) pyridine, trade mark Promintic) by intraperitoneal injection at the single dose of 200 mg/Kg,

Thiabenzadole (common name for 2(4′-thiazolyl)- benzimidazole) by oral route at the single dose of 250 mg/Kg,

Carbamazine citrate (common name for 1-diethylcarbamyl 4-methyl-piperazine citrate, trade mark Notezine) by subcutaneous injections at the single dosage of 20 mg/Kg,

Tetramizole (common name for 2-3-5-6 tetrahydro-6 phenyl amidazo (2-1b) thiazole hydrochloride, registered mark Nemicid) by subcutaneous injections a single dosages of 7· 5, or 15 mg/Kg and

Nivaquine (trademark for (4′-diethylamino 1′- methyl 1′-butyl) 4 amino 7-chloro quinoleine sulfate) usually employed as a coccidiocid, at the oral dosage of 9 mg/Kg during two consecutive days.

In spite of these difficultiies we think that research must be pursued in this direction. Meanwhile these initial results confirm that the specificity of several organophosphorus, not all, against larvae of warble fly is very high, although the metabolism of the drug in the animal body must be also taken into consideration.

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Recent Advances in the Control of Warble Flies

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Recent advances in the control of warble flies by insecticides is summarised. The possibility of controlling the parasite by the use of sterile males, insect attractants and other similar biological control methods are discussed.

The control of warble flies is an old and almost world wide problem. Scientific studies on these flies began in the 1st half of the 18th century. Yet it still remains a problem almost unsolved. While number of species of animals are infested by different species of warble flies the important ones among them are those which infest cattle, goats and reindeers. Those infesting cattle are called ox warble flies or cattle grubs and belong to two species namely, *Hypoderma lineatum* (De. Vill.) and *H. bovis* (L.). These are widely distributed in countries in the northern hemisphere. *H. bovis* is present in temperate region and *H. lineatum* extends into warmer regions such as the plains of India and southersn States of the U.S.A. Ox warble flies have recently been reported from a number of countries of Latin America namely Argentina, Chile, Ecuador, and Peru[15]. Another kind of Oestrid flies *Dermatobia hominis* (L.) popularly known as the 'tropical warble fly' infests cattle in some Latin American countries such as Brazil, Costa Rica and Nicaragua.

The importance of warble flies to livestock and livestock products (industries) in general and to the hides and skin industry in particular, is well-known. Attempts have been made, since long time back in different parts of the world, for the control and eradication of this pest.

The warble fly control measures so far adopted at different periods of time can be divided into 3 types. These are (1) the old method of manual or mechanical removal of larvae from warbles, (2) the chemical destruction of larvae in the warbles and (3) the chemical destruction of the larvae while in the process of migration through the body tissues of infested cattle.

The manual or mechanical removal of the cattle grubs from the warbles : During the early attempts for the control and even for eradication of cattle grubs the method employed was the removal and killing of the 2nd and 3rd instar larvae of the fly from warbles or swellings on the backs of the infested cattle. This removal was done by a variety of ways such as by squeezing the larvae out by pressing the swellings, by suction pump or by means of some sharp instruments. The classical attempts in the beginning of this century to eradicate the pest from some areas of Denmark was made this way, with the result the number of cattle grubs was reduced from 10 grubs per animal in 1901 to 0.1 in 1910. Similarly, in some parts of Ireland the number of cattle grubs was reduced from 11 to 4.2 per animal in one year. In the same country the pest was eradicated from the Clare Island, after 5 years by sqeezing grubs by hand[5].

The chemical destruction of the cattle grubs in the warbles : From the last decade of the 19th century, until recent years, attempts were made to kill the cattle grubs while in the warbles on the backs of the infested cattle with the help of chemicals. A large number of chemicals were tested for this purpose. The chemical was applied as injections, ointments, washes, sprays etc., on the warbles, so that it would reach the grub and kill it. By early 1920s, rotenone and a mixture of tobacco and lime were found to be the two most effective materials and the former became the standard drug for the control of warble fly in many countries such as Great Britain, U.S.A. and Canada. Rotenone was applied on the back of the warbled cattle 3 to 4 times during the warble season. The pest was very effectively controlled by the use of rotenone in the Isle of Wight in Great Britain and in Switzerland. In the Calumet Island in Canada cattle were treated 3 years from 1953, with 3 applications of rotenone annually. As a result of this treatment cattle grubs infestation was a little more than 2 per animal while the pretreatment number was more than 16 grubs per animal.

During 1948 to 1953 similar control programme was carried out with rotenone treatments in an area of 250 square miles in South Dakota in the U.S.A. This work was done in 175 ranches having altogether 20,000 cattle. On completion of the programme there were only 2.2 grubs per animal under treatment while more than 30 grubs per animal were found just outside the experimental area[14].

These projects showed that the reduction of the cattle grub infestation in the treated cattle was brought about to a very low level during the 1st few years, but reduction was not effective to eradicate the pest. The drugs such as rotenone, which kill the cattle grub while in the warble on the back of the cattle at the time of treatment, needed to be applied several times during the warble fly season, to kill the grubs when they come to the back, and the pest was killed only after the damage to the meat. For these reasons the stock owners did not show interest in adopting these control measures, thereby eradication of this pest, could not be effected even in countries such as Ireland, Great Britain and Canada, where warble fly eradication acts were in force.

The chemical destruction of the cattle grubs while in the progress of migration through the tissues of the animal body: Synthetic systematic insecticides are being tried in recent years. McGregor, Radeleff and Bushland[10] in U.S.A. demonstrated that some organo-phosphorus insecticides when administered to the warble infested cattle, would kill the cattle grubs while in the process of migration through the tissues of the animal body.

Since then a large number of organo-phosphorus compounds have been reported to be effective against this pest. Of these 3 compounds namely, Coumaphos, Trichlorphon and Ruelene have been found to be highly satisfactory and are being used in a number of countries. These systemic insecticides when administered by mouth, by injection, as sprays or as a wash, get absorbed into the circulation through the tissues and if the right concentration is used at the right time, were effective. The use of the systemic insecticides has a number of advantages over that of the older insecticides namely (1). it kills the 1st instar larvae of the pest before they damage the meat and hide ; (2). if used judiciously only one treatment a year is required ; (3) since the migration of the 1st instar larvae of the pest through the tissues of the animals takes several months there is enough time to treat a large number of animals ; (4) the treatment time summer and autumn is more convenient rather than the winter, when the application of rotenone is to be made ; and (5) with these treatments, if administered appropriately, 100% control of the pest can be achieved.

The result of experiments carried out in an isolated range herd in British Columbia, Canada between the years 1957 and 1965 have been reported[12]. There were about 1,000 cattle in the herd distributed over an area of 200 sq. miles. Fenchlorphos was used for treatment during the 1st 4 years and Ruelene in the 5th and 6th years. The 1st two treatments reduced the infestation in the cattle to a great extent, the grubs being only 1.6 per untreated

calf whereas it was 30.2 grubs per animal prior to treatment. In the 6th year the infestation appeared to have decreased below its biological potential to maintain itself (i.e. to 0.2 grubs per untreated calf). In the 7th year the infestation persisted at approximately the previous low level and in the absence of insecticidal treatment the infestation increased to 10.2 grubs per animal subsequently.

Riehl *et al* [13] have reported that in the treatment commenced in 1960 and one treatment with Ruelene was given to the animals each year by the pour on method and the infestation was found to have progressively decreased from 22.6 per animal in 1960 to zero in 1963.

Popov *et al*[11] report that over the entire country of Bulgaria the proportion of warbled cattle had fallen from 21% in the period of 1955 to 1959 to 6% in 1959 to 1965 as a result of the introduction of treatment of cattle with trichlorphon. The proportion of production of 1st class hides has risen from 72% to 84%.

In Techlenburg area (Germany) trichlorphon suspension was applied to all cattle of at least two years of age between mid November and early December starting in 1963. By 1966 only 1% of the treated animals showed a small number of warbles on them[9].

The most extensive programme for ox warble eradication was started in 1963 in Ireland. By 1966 more than 5 million animals *i.e.* almost all cattle in the Republic of Ireland were treated with either Reulene or Dyvon (a trichlorphon formulation). During the period of March to June 1966 out of 44,998 cattle exported from Ireland *via* Birkenhead to England only 82 were infested with cattle grubs whereas prior to the commencement of the programme 40% to 60% of the Irish cattle passing through Birkenhead were infested with the pest[5].

It is thus evident that the effective control and even eradication of ox warble flies, from geographically isolated areas, may be possible with the use of the systemic insecticides. In fact, the pest has already been practically eradicated from some such areas *viz.* the Inishowen Peninsula (county Donegal) in Ireland and the Isle of Man in Great Britain[2].

In some countries, particularly in America, attempts are being made to use biological control methods along with systematic insecticides for the control and eradication of cattle grubs. These biological control methods are (1) the use of sterile males, (2) the use of insect sex attractants and (3) immunization.

The use of sterile males : The principle involved in this method for the eradication of the insect pests is, that if a large number of sterile males of a given species of insect can be released in the field they will mate with the normal females and the resulting eggs will be infertile. The sterility in insects can be induced by their exposure to X-ray or gamma ray. In recent years some chemosterilants are being used for inducing sterility in insects. This method was first successfully tried in the field in the West Indies Island of Curacao in 1954[1] and then in the south eastern states of the USA[8] against the screw worm fly *Cochlionyia hominivorax* (Coquerel). The method had subsequently been tried against a number of other insect pests but success was not attained in every case. The method is likely to be effective in cases where (1) the natural population of the pest species is low, (2) the females mate only once in its life, (3) males are easy to rear in large numbers and (4) chances of migration of the species from outside into the area under operation are nil. Experiments are being carried out in some places to obtain certain information about the pest with a view to use this method for the eradication of warble flies. Drummond[4] succeeded in inducing sterility in *H. lineatum* by exposing pupae to gamma radiation. But notwithstanding the fact that now it is possible to produce flies, eggs and newly hatched larvae of cattle warble flies under controlled conditions[1,6,3] attempts to rear their larvae *in vitro* have not been successful so far. If an easy method for producing sufficiently large number of sterile male warble flies is found out it may be possible to eradicate the pest by the use of sterile males. (after the fly population is reduced to a low level by the use of systemic insecticides for a few years.)

Insec sext attractants : Insects are known to be attracted by chemical substances produced by their mates particularly by the virgin females. In the recent years such chemical substances have been isolated, and used for the control and eradication of a number of insect pests[6]. As Graham and Drummond[5] have remarked because of their great potential for use in the elimination of the small numbers of survivors of an insecticide treatment programme, sex attractants in *Hypoderma* should be studied.

Immunization against cattle warble flies : Khan *et al*[7] made an attempt to control cattle grubs by preparing two types of antigens which they called antigen A and antigen B. The antigens were prepared from the 1st instar larvae of the common cattle grub *H. lineatum* obtained from slaughtered cattle. A larval homogenate containing 10 larvae per ml of saline was prepared and designated as antigen A. This homogenate was treated with tannic acid and the resultant precipitate washed thrice in saline and restored to its original volume. This was termed antigen B.

To one group of about 20 calves antigen A was injected intramuscularly, and 17 days later antigen B was administered in the same manner. To another group of calves antigen A alone was injected. It was found that the number of northern cattle grubs (*H. bovis*) was reduced by the injections significantly. Treatments 1 and 2 reduced the number of grubs by 81% and 48% respectively. But there was no reduction in the number of the common cattle grub (*H. lineatum*) in the calves in these trials. The work should be continued to develop an effective vaccine against the cattle warble fly infestation. However, this can be done profitably only after a technique for rearing the larvae of the pest under controlled condition *in vitro* is evolved.

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Influence of Pox Lesions on the Characteristics of Sheep and Goat Skins

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Tanned sheep and goat skins marked with pox lesions are classified as rejections and they constitute a great loss to the export trade. An incidence of this defect ranges from 5-20% in different areas resulting in considerable loss.

A critical study of the pox affected areas of leather has been made, in relation to physical properties and their affinity for dyes. Tensile strength of strips of leather including pox marks were not significantly different from adjacent normal strips, while bursting strength of the affected areas were lower than that of normal ones, and the affected areas were also more permeable to dyes.

Amongst the viral diseases affecting Indian livestock, pox, particularly in sheep and goats may be described as the most deleterious one to skin quality. The blemishes produced on the grain side of tanned leather are quite obvious and account for considerable depreciation of their foreign exchange value, they being graded as double rejections by the exporters.

Pox, caused by the filterable virus "*Variola ovina*" in sheep and "*Variola caprina*" in goats, has a profound influence on the structure of the skin. The pathological changes, though primarily seen in the cell layers of the epidermis, being an epitheliotropic virus, the associated changes produced on the dermis and grain results in permanent scars. The changes in the histological changes of the skins during the course of the disease has been described by earlier workers[1-6]. From the leather manufacturers' point of view, skins recovered from animals after an attack of pox, revealed circular areas on the

grain surface. These appeared as light circular areas on the flesh side and circular scars on the grain side of the leather. *Infectious dermatitis* named as a typical goat pox also results in skin blemishes[11]. It is cited, that pox affected the skin quality, the prevalence in them being often greater than one percent in sheep and goat skins and that they were clearly visible on the flesh side of these skins as discrete circular spots[3]. While the pox lesions may lead to twisting of the grain layer[10] they resulted in white spots on the flesh side of tanned leather[1,6]. An inquiry into these skin conditions in Indian sheep and goat skins showed that the percentage incidence was much higher and may even rise up to 50% in sheep skins, during epidemics[8].

In a study of the physical properties of normal leather, Muthia *et al*[9] have reported earlier that tensile strength of leather varied according to its location such as butt, belly, sides, etc. The present communication relates to the influence of pox marks on certain skin characteristics.

Materials and Methods

Skins obtained from known pox infected animals as well as those recovered after an attack of pox have been used in these studies.

Examination of freshly flayed and cured skins: Sheep skins 3,200 in number and 750 goat skins were examined in the Saidapet slaughter house, covering different seasons of the year. A large number of salted market skins were also examined with a view to get an idea of the seasonal prevalence.

Examination of tanned skins : Random lots of 100 skins each were examined at the time of assortment of tanned skins. The number showing these defects as the percentage of total number examined is given here. Care was taken to ensure that the tanner drew his raw material from the same source.

Influence of Pox Lesions on the physical properties of leather : For this purpose known pox affected skins were obtained and tanned by standard vegetable tanning process. Tanned skins were examined for the following physical properties.

Tensile strength : For this purpose strips of leather 1 cm in width and 11 cm long were cut from affected skins, since the pox lesions usually had a diameter of 7-8 mm. The lesions were located in the middle of the strips. Adjacent strips on either side of identical size but without pox lesions served as controls. A number of such lots of skins both perpendicular as well as

parallel to the backbone line were examined. The test pieces were clamped in such a way that the pox lesion was in the middle of the jaws of the tensile strength recording machine. Forty-three affected samples and eighty six controls were tested and values obtained were expressed as Kg per cm^2.

Bursting strength : Bursting strength studies were made with the pox lesions in the centre of the test area. This was compared with normal area without the defect, just anterior and posterior to the affected area. These samples lateral to the affected areas were also tested similarly. Thus it will be seen that each affected sample was compared with four normal areas adjacent to the same.

The data obtained both in cases of tensile and bursting strengths studies were analysed statistically to determine whether the values of normal areas were significantly different from those of affected ones. The statistics followed was that for paired samples the null hypothesis assumed being that the mean difference or advantage of the normal sample over the affected one is 0, the formula used being

$$t = \frac{x - \mu}{S / n} \text{ where } \mu = O$$

Dye permeability: These studies were conducted in an apparatus designed for this purpose. The apparatus consisted of flanges made of brass held tightly by a threaded cap. The samples were held with the grain side up, the affected leather in opposition with a hole on the flange in the centre. The hole was 3 mm in diameter so that the piece of leather in opposition with the hole permitted the flow of the dye through the pox affected area or normal area of leather. The dye solutions were made to pass through the circular area of leather at a constant hydrostatic pressure of 261.5 gm/sq cm by a suitable arrangement. Care was taken to see that samples of identical thickness, that is 0.8 mm (0.78 mm to 0.82 mm) were used.

A 0.1 % solution of the respective dyes were used in these tests. The dye solutions were allowed to pass through and the filtrates received after passage were collected after specific time intervals and were estimated calorimetrically to determine the concentration of the dye. The *p*H of the dye solutions used were adjusted to 7.4.

Results and Discussion

The skins on an examination of the flesh side revealed discrete circular spots being of a whiter colour as compared with the surrounding areas. On a closer scrutiny, corresponding areas on the grain side of the skin revealed bald patches. Animals with active pox lesions on the skin were not encountered in the slaughter houses, and hence the lesions examined mainly consisted of healed pox marks. The percentage prevalence of pox lesions in the raw sheep and goat skins is given in Fig 1 and 2.

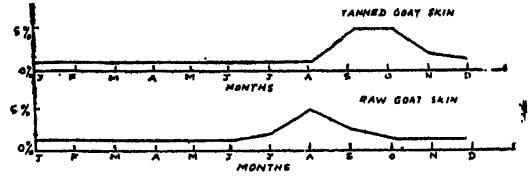

Fig. 1. *Percentage incidence of goat pox during a year*

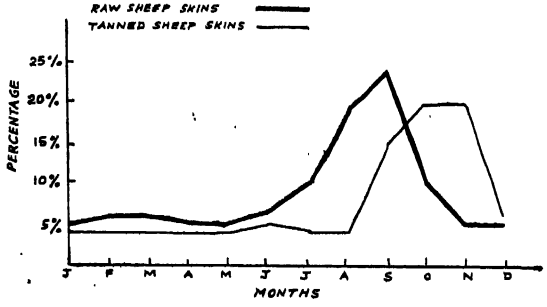

Fig. 2. *Percentage incidence of sheep pox during a year*

It will be seen from the graph that the presence of pox affected skins remained at 5% level during the major period of the year. During the months of June, July and August the number of such affected skins rose to as high as 20-25%. While the maximum extent at peak periods to which goat skins were infected never exceeded 5%.

Vegetable tanned skins numbering to 4,100 sheep and 3,200 goat skins were examined in tanneries. The percentage of pox defects revealed a similar trend to that of raw skins. Depending on the district or block of the region, pox ranged from 5-20% in sheep and to a maximum of 5% in goats skins during a year. In the tanned skins the defect appears to reach a peak period during the months of August, September and October, vide Fig 1 and 2.

Tensile strength of twenty pox and normal samples are expressed in kg per sq cm in Table 1. Statistical analysis of the data revealed that the pox affected samples were not significantly different from normal skins tested under the same conditions. Further, studies on the point of break, showed that they were random with no particular relationship between the pox lesion and breaking point in pox affected samples. The initial length of all samples were 8 cm clamped between the jaws. The average maximum stretch of forty normal samples was 13.22cm and the point of break measured while in stretched conditions was 7.04. The average maximum stretch of twenty pox lesions were 15.902 and the breaking point 8.04 cm.

TABLE 1

Tensile Strength — Pox—Normal

Range of values

No of samples tested	Ts of pox sample Kg/sq cm	Ts of adjacent normal sample Kg/sq cm	Relative increase in in Ts (X)	X^2
1	76.17	84.24	+8.07	65.1249
2	96.39 to 105.63	94.54 to 127.69	—1.852 to + 22.66	3.4255 to 486.6436
3	113.40 to 118.93	128.86 to 134.78	13.46 to 15.85	231.0116 to 251.225
2	133.75 to 134.49	108.78 to 190.33	—24.97 to + 56.04	623.5009 to 3340.4816
5	154.88 to 166.68	204.64 to 162.74	+49.76 to — 3.94	2476.0576 to 15.5236
4	177.21 to 198.02	177.41 to 199.26	0.10 to + 1.24	0.01 to 1.5376
Total	2749.74	3006.05	256.31	18390.8545 Ex^2
Mean	137.49	150.30	1.2815	...

Total number of tests carried out 17

The studies on bursting strength conducted, comparing the anterior and posterior sides with the affected sample, and the adjacent sides with the affected pox sample gave different results. The results are presented in Tables 2 and 3.

TABLE 2

Bursting strength of leather of pox affected area compared with anterior and posterior normal area

No of samples tested	Range of values		X	X^2
	Mean of anterior and posterior Kg/sq/cm	Pox sample Kg/sq/cm		
1	110	127	17	289
11	146—183	166—175	17—29	261—289
14	195—239	222—235	4—32.5	16—1056.25
5	246—300	222—262	16—78	256—6.84
Total	6384.5	6426	88.3	29732.25
Mean	205.95	207.2903		

Total number of tests carried out — 31

TABLE 3

Bursting strength of leather, pox affected and normal adjacent area 3 inches away from pox lesion

No of samples tested	Range of values		X
	Pox Kg /sq /cm	Normal Kg /sq /cm	
1	111	178	+ 67
2	125—127	188— 18	+135— + 61
8	157—185	192—200	+135— — 15
11	190—228	175—262	— 15— +134
9	235—272	205—238	— 30— — 34
Total	6426	6888	636
Mean	207.2903	222.1935	

Total number of tests carried out — 31

Pox samples compared with nearest anterior and posterior normal skins reveal that the affected samples were not significantly different from the normal ones. From Table 3, the value of 't' for 30 degrees of freedom at 5% level is 2.042 Since 0.5063 is smaller than 2.042 (determined by using the formula mentioned earlier), difference in the bursting strength of adjacent tissues is not significantly different from pox sample.

From Table 3, the 't' value can be calculated to be t=2.42 and freedom at 5% level. Hence the difference is significant.

It will be seen from the graph (Fig. 3) that the rate of flow of dye in known time intervals is greater in the affected tissue than normal in experiments with acid aniline blue. In the case of methylene blue the difference does not appear marked (Fig. 4). The colorimetry of dye collected

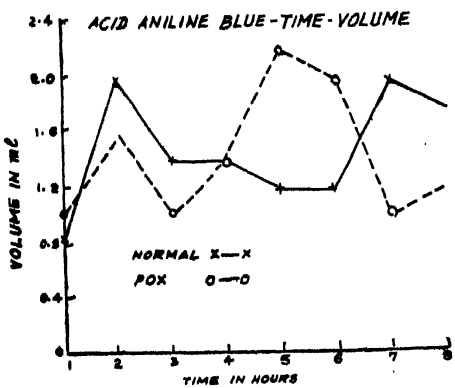

Fig. 3. *Rate of flow of acid anilne blue dye solution through normal and affected pox area during an eight hour period.*

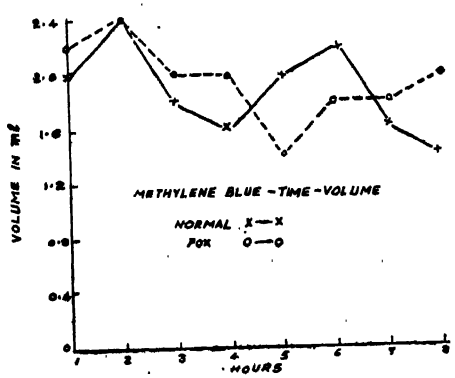

Fig. 4. *Rate of flow of methylene blue dye solution through normal and affected pox area during an eight hour period.*

at the free end reveal defintely a greater retention of the dye by normal as against the affected tissue. With acid aniline blue the results do did not show great variations (Fig. 5).

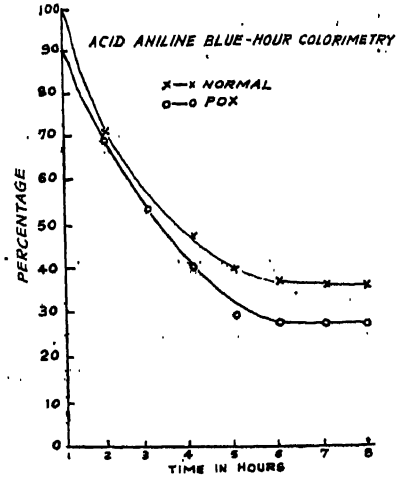

Fig. 5. *Percentage transmission of light at 475 mμ of the dye collected from pox and normal sample passage during eight hour period.*

The presence of pox in the tanned skins or skin received in the tannery may not correspond with the presence of outbreaks in the districts, the reason being that animals showing active lesions are not brought for slaughter. Recovered animals though they have overcome the disease still show these marks.

Sale of animals after a particular age, food habits and slaugtering practices in the regions may therefore account for this seasonal incidence. It is also seen that the incidence in tanned leather followed that of the raw skins, with a lag period of about two months. This may be accounted for by the time lag between procurement and tanning of these skins in the tannery.

It is generally believed that the region in the skin with scar mark is usually weaker than the surrounding tissue. The point of break in the T_s determinations did not in the majority of cases (16 out of 20) occur on the pox lesion and it was clearly away. Hence, it appears that the healed pox mark was quite strong as compared with surrounding normal tissue. However, from bursting strength point of view the pox marked tissue were lower or similar by the two methods applied in the test.

The pox lesions in the leather was mostly composed of fibres arranged parallel to the surface. While the normal corium was characterised by a three-dimensional weave pattern, composed of fibre bundles. Such a compact arrangement of fibres in the pox lesions was expected to interfere with the permeability to dye solutions. The present studies indicate that pox affected areas of leather were more permeable to dyes than normal tissues. Studies on affinity fixation of dyes, and appearance of white spots on the flesh side of pox lesions require further investigation.

Acknowledgment

Our thanks are due to the Indian hides and skins improvement society, under which the work was undertaken, and Dr. Y. Nayudamma, Director Central Leather Research Institute for the facilities granted, and the advice and guidance in carrying out the work.

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DISCUSSION

SESSION IV

C.M. Singh (*Indian Veterinary Research Institute, Izatnagar*) : The affections of the skin described by Dr. Bertie D'Souza may be mainly divided into two categories (i) affections of skin which directly damage the skin and (ii) indirect causes like parasites, etc., which cause an indirect loss.

In one of the raw material committee it was estimated that 20 crores worth damage is done by misutilisation of slaughterhouse byproducts and 200 crores by the nonutilisation of fallen carcasses. Is there any estimate of the losses incurred due to (i) improper handling of hides and skins (ii) due to not utilising the carcasses of animal dyeing in goshalas and go-sadhans.

Y. Nayudamma (*Central Leather Research Institute, Madras*) : A lot of information is available in relation to the estimates as to the losses occurred at various stages in different reports. Is the wastage of hides and skins due to want of research, money or practices? If we have enough knowledge why not we start practising it ?

Bertie A. D'Souza (*Madras Veterinary College*) : It is the improper liason between the researcher and farmer which is responsible for the lacunae. There are different problems posed by different areas which have to be worked out. The local research should be encouraged and developed. Priority is not given to local research. Financial assistance is needed for this.

R. Bhaskaran (*Central Leather Research Institute, Madras*) : All the diseases of the different areas should be studied on a more systematic and detailed way. Most of these diseases are spread over wide areas. Unless control measures are taken up over such wide areas, simultaneously, the effect of such a treatment will not be felt significantly.

C.M. Singh: The present researches bore in mind only the losses due to morbidity and mortality of animals. So far the research in our Institute or elswhere regarding the diseases of animal has not been planned so as to take into account the losses incurred due to the diseases directly or indirectly on the leather industry. For instance, " Ringworm" is a very common infestation in animals all over India, but nobody knows the amount of losses caused by this disease to the leather industry. " Ring worm " and other skin infections cause thickening of the skins. Do these thickenings have a detrimental effect on the hide quality ? What is the extent of damage caused by mange to the leather industry ?

R. Bhaskaran : About 5% of the skins are affected by fungal and other skin diseases. Ringworm results in circular scars which reduce the quality of skins to rejection grade. There are certain difficulties in collecting statistical data on these losses. Demodectic mange in the nodular form in which it usually occurs in goats affects nearly 5-20% of these skins. These nodules produce pin holes on the grain and white spots on the flesh side. The skins are unsuitable for suedes and when too many pin holes are present they are rejections.

Y. Nayudamma: Dr. Chandrasekaran is perhaps aware that CLRI is collaborating with medical experts in the electron microscopic studies of skin tumours of humans. A similar study of the tumours of animals can also be undertaken.

K.P. Chandrasekaran (*Madras Veterinary College*) : Yes, such a collaborative study can be made.

R.P. Choudhury (*Indian Veterinary Research Institute, Izatnagar*) : The paper of Messers Divakaran and others is interesting. I wish controlled experiments are done, taking animals infesting them with the longirostrate and brevirostrate ticks to confirm the above findings. Otherwise this publication will not bring this to light.

R. Bhaskaran : In these studies the skins were obtained with the specific ticks *in situ* and the ticks were collected and classified as longirostrate and brevirostrate types. Here the lesions relate to those caused by adults (mostly females) of *Hyalomma, Boophilus* and *Haemaphysalis* species. To this extent the types of lesions caused and the defects seen in the leather were confirmed. However, controlled experiments are necessary to determine the damages caused by the different developing stages of specified species of ticks.

S. Divakaran (*Central Leather Research Institute, Madras*) : Regarding controlled experiments contact is being made with Madras Veterinary College to do this work. During our work replica charts of the skins as to the distribution of the 2 types of ticks were made to make ourselves sure which type of tick is present in the respective re ions examined.

Y. Nayudamma : Proper collaboration between Animal Husbandry Department, Central Leather Research Institute and the Indian Hides and Skins Improvement Society should take steps to eradicate the cause of the white spot defect.

INDIAN LEATHERS

Leather and allied industries rank fifth among the materials which our country exports and accounts for nearly 5.8% of India's total foreign exchange earnings.

India has the largest number of domestic animals in the world and produces enormous quantities of skins and hides. India is the largest exporter of leathers in the world.

The world famous East India (E.I.) vegetable tanned skins and hides are the products of an ancient craft practised and perfected down the ages. Some of the unique properties associated with these leathers are, fine texture, strength, good smooth grain, absence of flay and other cuts, firm flesh giving good nap, mellow and uniform and light tannage, high tensile strength, etc. E.I. tanned hides and skins valued at Rs. 347.75 millions were exported during the year 1967–68. Besides this, India also exported pickled goat skins, tanned reptile skins goat hair and tanning materials valued at Rs. 51.48 millions.

The Leather Export Promotion Council has been sponsored by the Government of India in order to support, protect, maintain, increase and promote the exports of (i) tanned hides and skins (ii) bye-products of the tanning industry such as fleshings, goat hair and (iii) tanning materials. For further details, please write to :

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